Dr. Geo. W. Beach — Food Flavors

CHEMISTRY of NATURAL FOOD FLAVORS

for use in setting up flavor-odor panel.

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— a Symposium

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Chemistry of natural food
flavors

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CHEMISTRY OF NATURAL FOOD FLAVORS

A symposium sponsored by the

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May 1957

SURVEYS OF PROGRESS ON MILITARY SUBSISTENCE PROBLEMS

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SERIES I. FOOD STABILITY

1. Contributions of Browning Research to Ration Item Stability
2. Stability of Shortenings in Cereal and Baked Products
3. Stability of Dehydrated Eggs
4. The Quality and Stability of Canned Meats
5. Color in Foods
6. Dry Whole Milk
7. Precooked Frozen Foods
8. Yeast, Its Characteristics, Growth and Function in Baked Products
9. Chemistry of Natural Food Flavors

SERIES II. NUTRITION ASPECTS OF RATIONS

1. Nutrition Under Climatic Stress
2. Methods for Evaluation of Nutritional Adequacy and Status

SERIES III. FOOD ACCEPTANCE

1. Food Acceptance Testing Methodology

SERIES IV. MILITARY UTILITY OF FOODS

1. Establishing Optimum Conditions for Storage and Handling of Semi-perishable Subsistence Items

Opinions expressed in the symposium on *Chemistry of Natural Food Flavors* are those of the individual participants and do not necessarily represent the views or policies of the Department of Defense.

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Proceedings of the Symposium on Chemistry of Natural Food Flavors

I. INTRODUCTION—THE EMERGING SCIENCE OF FLAVOR

COLONEL JOHN D. PETERMAN

Commandant, Quartermaster Food and Container Institute for the Armed Forces

It is indeed a pleasure for me to welcome you to our conference on the chemistry of natural food flavors. A meeting such as this which brings together people who have an interest in and a knowledge of various aspects of flavor research should be stimulating to creative thinking on some of the problems which face us in our efforts to provide improved foods for the Armed Forces. This meeting should help to give us a great deal of information on the progress made to date on flavor research, allow us to orient our present position, and plan attacks which will result in profitable advances in the future.

We feel that the subject of today's conference is one of interest not only to the Armed Forces but to the Food Industry as well. It is believed that in the years ahead food processors will place increasing emphasis on improvements in the flavor quality of their products.

The chief problems in this area of flavor have to do with the prevention of loss of the desirable flavors which may be brought about by processing and storage, and, in some cases, the use of processing to increase the flavorful factors in foods. Many foods are eaten only after processing treatments which profoundly affect their flavor. Sometimes the changes which occur are highly desirable, and the food is converted from an unacceptable to a highly acceptable item. Examples are the roasting of green coffee, the roasting of peanuts, the baking of bread, the roasting or cooking of meats. On the other hand, certain treatments used for the purpose of processing foods sometimes have an adverse effect on their quality. For example, during canning of meats, substances develop which lower the acceptability of the items; during irradiation of some foods, such as beef, adverse flavor changes occur; during dehydration of some foods, such as cab-

bage, volatile flavor components are lost. Although these are only examples, they are indicative of the challenging nature of the problems in this area.

We need to know the characteristics of highest flavor quality in various food items; we need to know how to achieve this quality and how to preserve it. With the availability and application of our most advanced chemical and physical techniques and processing knowledge, it seems reasonable to expect that progress in the next 10 to 20 years can be expected to be much more rapid than in the last decade. There appears to be the distinct possibility that the acceptability of certain food items might be significantly improved by fortification with flavor substances. For example, perhaps it will be possible to enhance the acceptability of some of our meat items, soups, and gravies once we know the chemical nature of desirable meat flavor compounds and they are synthesized. It is well known that soluble coffee does not possess the aroma characteristics of coffee freshly brewed from the grounds because the volatile constituents are lost. It is also well known that the industry is actively engaged in research on this problem. We face somewhat similar problems in regard to the loss of volatile flavor compounds during dehydration of various food products. Can these losses be prevented? Can the volatile flavor substances be captured and returned to the dehydrated food? Can certain enzymes acting upon precursors regenerate the lost flavor? The answers to these and many other questions can come only through research.

I wish to thank the speakers who are appearing on our program and, again, I want to welcome all of you to this conference.

Interest of the Quartermaster Corps in Flavor

DONALD K. TRESSLER

Scientific Director, Quartermaster Food and Container Institute for the Armed Forces

The acceptance of food is determined principally by the color, texture, odor, and flavor. Of these, flavor and odor are by all means the most important criteria of quality and largely determine whether or not a food is eaten with pleasure. If the flavor of a food is so poor that it is not eaten by the soldier, it makes little difference what its nutritive value is, for the food is wasted.

Food of poor flavor is the cause of a large proportion of the soldier's gripes and is often the cause of low morale. Food of good quality, carefully prepared and cooked, has good flavor and

is eaten with relish. The soldier who eats it will be well-nourished and should have high morale.

The Quartermaster, as Single Manager, now buys the food, not only for the Army but for the Air Force, the Navy, and the Marines. The Navy and the Air Force are just as particular as the Army about food quality. Seamen in the cramped quarters of submarines are greatly concerned with the flavor of the food served. If their meals are not excellent, the seamen will suffer boredom on long voyages, because of the dull monotony of the situation.

Airmen on long, arduous flights are tense and will not relish their food if its flavor is uninviting. Here at the Quartermaster Food and Container Institute, we are working on the improvement of in-flight rations, and we must learn how to evaluate and improve their flavor.

Is it any wonder, then, that the Quartermaster is concerned with the flavor of the food it buys and serves? As a matter of fact, the Food and Container Institute is cooperating with the Pioneering Laboratories, of the QM Research and Development Center, Natick, Massachusetts, in a broad program designed (1) to determine the chemical nature of most foods; (2) to measure food flavors developed during processing; (3) to study the effect of various unit processes and combinations of processes on food flavor; and (4) to enhance the flavors of processed foods by the addition of enzymes and enzyme preparations. This last development is expected to effect flavor improvement by the action of enzymes on the naturally occurring flavor precursors.

This symposium is being held for the purpose of determining and critically evaluating our present knowledge of the flavors of fruit, vegetables, meat, poultry, cheese, bread, coffee, and a few other foods. We also will hear papers concerned with estimation of flavors and hope to get some suggestions concerning the most modern methods and techniques used in flavor research.

The information and suggestions offered here should be of great help in planning our new program.

The Importance of Flavor Research

EMIL M. MRAK

Chairman, Department of Food Technology, University of California

The realization of the importance of flavor and the willingness to consider it from the standpoint of the chemical nature of the substance involved is a relatively new concept. While much has

been said about flavor, the serious consideration of it occurred only when acceptance testing became a well defined and accepted procedure during World War II. Consideration of the chemical aspects of flavor has been undertaken by relatively courageous scientists. Apparently, the chemical approach is so complicated and involves such difficult techniques and so much time that it has frightened away even very good chemists. We now have reached the point, however, where we must overcome this fear and look at the chemical nature of flavor compounds more seriously.

Man's early concern with food, largely from the standpoint of sufficiency, has been first in obtaining enough and then finding some means of preserving it. In accepting available procedures of preservation such as drying or salting, he naturally had to accept changes in flavors entirely different from those of the fresh food. Because of the need for food and a willingness to accept foods preserved in any way they could be preserved, off-flavors were accepted but probably would not be accepted today.

A look into the history of troop feeding during the Napoleonic Wars, the Civil War, the Spanish American War, and even World War I, indicate that the important factors with respect to food were preservation and distribution of foods. It was considered an achievement if the troops could obtain sufficient edible foods to keep them going. This is well indicated by Napoleon's great desire to obtain a means of preservation and also by the stories of embalmed meat that were prevalent after the Spanish-American War.

According to Dr. Dove, in the United States there has been during the past 75 years a movement of people from the farm to the city. When living on the farm, individuals had the opportunity to decide on the types of food preparations and even raw materials that would be used in the foods. Each family sitting at the dining table was an acceptance panel. However, as this population moved into the cities, the situation changed drastically because of urban living conditions and the increased population was compelled more and more to resort to processed foods. While taste may have been important, it was a factor that had to be overcome by the consumer, and he was compelled to accept foods that were certainly different than those he was accustomed to in the home. In other words, as these people moved into the cities, new markets for processed food developed and the processing of foods increased. During the early stages of this development, processors gave most intensive consideration to factors of preservation rather than flavor. The consumer had no choice but to

develop an appetite for these foods or perhaps find himself in a handicapped situation.

As time went on, the preservers of food became concerned with other problems such as deterioration caused by chemical, enzymatic, or microbiological activities. Later problems relating to color, nutritional value, and even the aesthetic values were considered. The serious consideration of flavor, however, has been slow in coming, and then largely by taste panel testing.

Today, we are told, the sale of processed foods exceeds that of fresh foods. The food processing industry is a large one, and the competition is great for the consumer's dollar. It might be said therefore, that the consumer is in a position of dictation. The shoe is on the other foot! In view of this, the processor has found it necessary to give more and more consideration to the consumer demand. The Army has been aware of this and has tried to give the Soldier Consumer the most acceptable foods possible.

This has required consideration of factors of acceptability including color, texture, and taste as determined by taste panels, the dramatic development of convenience foods, and consideration of stability and shelf life.

As indicated above, the aesthetic and nutritive values also have received serious consideration. It is needless to say that the processor has done a remarkable job in making processed foods safe. As a matter of fact, I believe the food processors have made tremendous advances, and they are to be complimented on the excellent progress they have made in recent years.

Now that food science is a well established area of activity and one that has replaced the art of food manufacture, many basic studies relating to the chemical and physical characteristics of foods are underway. One need but visit laboratories in various areas of the world and he is impressed with the number and variety of fundamental studies on foods that are under way. One also is impressed with the fact that relatively few studies are devoted to the nature and chemistry of flavors. It is, however, very heartening to note that some of the largest manufacturers of foods are devoting more and more time to the nature of the flavor factor. What are the flavors in poultry and meat, fruits and vegetables? How do they change during processing or cooking or handling of the particular commodity? What is the chemical nature of these flavors, and how stable are the flavors? What blends of flavor occur in a particular food? How may these flavors be produced and added to foods to make them more acceptable to consumers? These are some of the questions that I

know are being considered by some of the food industries. I believe that this type of work must be expanded, for it is one of the most important and perhaps most neglected areas in food science today. The Army group is to be complimented on sponsoring a symposium in this important field. Perhaps this symposium will reveal what we should do rather than merely review what has been done. In the past the Quartermaster has sponsored other symposia in very important areas. A notable one was concerned with color in foods. There is no question in my mind but what this has stimulated a great deal of scientific work in this area. It is my hope that this symposium will stimulate interest and activity in the basic study of the substances responsible for the flavors of foods. We cannot progress in the area of food acceptance if this is not done.

II. TECHNIQUES AND METHODS FOR RESEARCH IN FLAVORS

Facts and Theory on the Mechanism of Taste and Odor Perception[1]

LLOYD M. BEIDLER

Professor of Physiology, Florida State University

It is appropriate that the mechanisms involved in taste and odor stimulation should be considered in this Symposium on the Chemistry of Natural Food Flavors. Flavor is usually a composite of taste and odor, although the senses of pain, touch, warmth, and cold also may contribute (fig. 1). The chemist

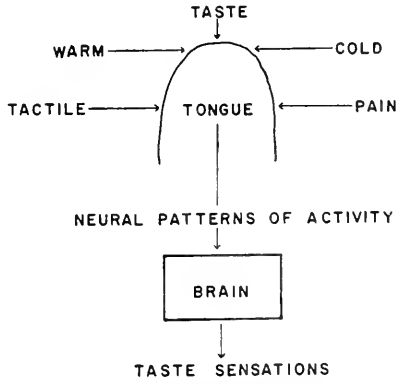

Figure 1. Taste sensations depend upon the responses of many different sense organs.

interested in food flavors would naturally like to have a well-organized treatise on the physiological and psychophysical basis of flavor perception, but none exists. There is no satisfactory theoretical basis of taste and smell on which a food technologist might rely, but the literature reveals comparatively few studies of the senses of taste and smell during the past 30 years.

[1] This work was supported in part by a contract between the Florida State University and the Office of Naval Research, the Office of The Quartermaster General, and the Armour Research Foundation.

The results of a few recent studies on taste and smell are presented in this paper. Emphasis is given to objective and quantitative data obtained from experimental animals. From these data several generalizations concerning the mechanism of taste and olfactory receptor stimulation are suggested.

Taste

The Morphology of the Taste Structures

The receptors associated with taste are usually found in the taste buds. These end organs first were found in fish in 1851 and were not associated with the function of taste until 1863. Four

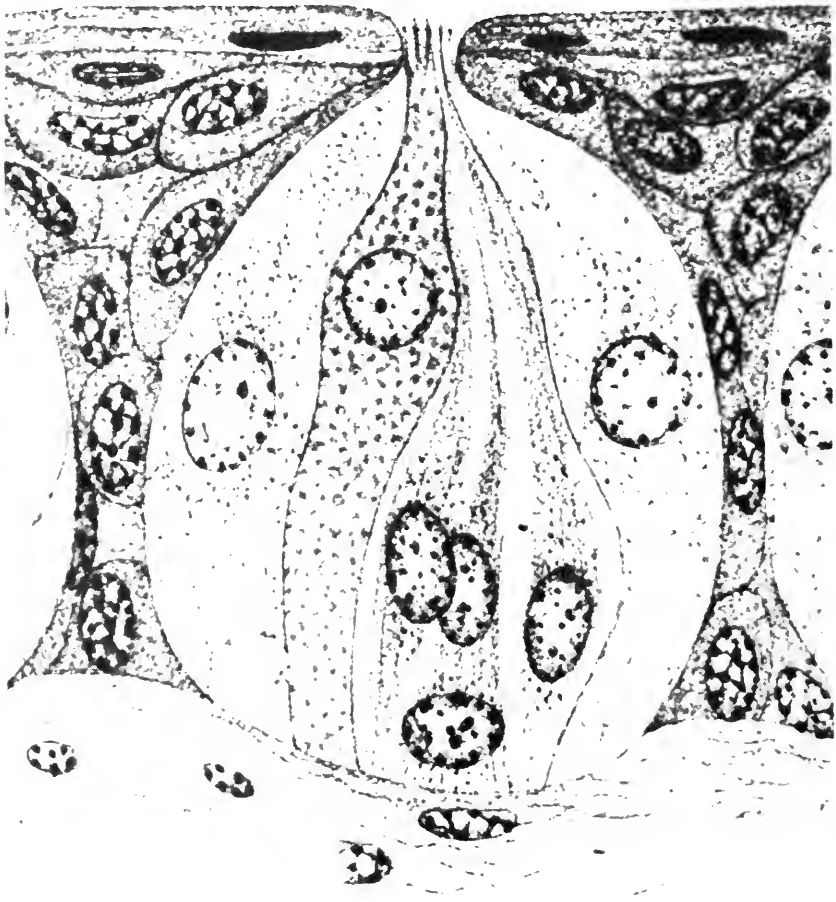

Figure 2. Section through the foliate papilla of a rabbit showing taste and so-called supporting cells. After Lenhossek (26).

years later Schwalbe *(23)* first described taste buds in man. The taste buds are composed of two types of cells, a supporting cell and a taste cell. Both types of cells are grouped together into a small bud-like structure, the taste bud (fig. 2). The number of mitotic divisions and the presence of leukocytes observed in histological sections of taste buds led some to believe that the older taste cells are constantly replaced by new taste cells.

A narrow passage, the taste pore, connects the taste bud to the open surface of the tongue. Electron microscope studies show 3–5 villi-like projections 3–5 micra in length and 0.2 micron in diameter entering the pore from each taste cell *(12)*. Many believe that these structures are stimulated by the substances that are tasted. These fine projections should be analyzed for their chemical constituents, but the very small size and number have made such an analysis extremely difficult. The taste bud is innervated by small nerves that arise from a subepithelial plexus, wind around the taste cells, and terminate in knoblike projections on the cell.

The number and spatial distribution of taste buds vary from one species of animal to another, but the bud size remains fairly constant, with a diameter of about 30 micra and a length of about 60 micra (table 1).

Table 1. Circumvallate papillae

Species	Number papillae	Number taste buds	Bud length micra	Bud diameter micra
Opossum	3	2,900	54	34
Wombat	3	3,500	65	30
Armadillo	2	2,500	51	30
Jack rabbit	2	1,200	49	30
Prairie dog	3	1,100	52	30
Chipmunk	3	750	51	29
Bat	3	3,500	60	29
Pronghorn	52	48,000	69	32
Otter	7–8	2,400	53	30
Timber wolf	2	2,900	60	31
Coyote	7	5,000	58	33
Dog	4–7	8,000	65	35
Rhesus monkey	3	1,800	68	36

From Tuckerman *(25)*.

The taste buds are found primarily on the dorsal surface of the tongue, although they may be sparsely distributed over other structures of the oral cavity. On the tongue they are located in the trenches of the circumvallate papillae, in the grooves of the foliate papillae, and above the dermal core of numerous fungi-

form papillae. The taste solutions normally enter the grooves and trenches by convection currents generated by the muscular activity of the tongue. The anterior two-thirds of the tongue contains only fungiform papillae (fig. 3), and is innervated by a

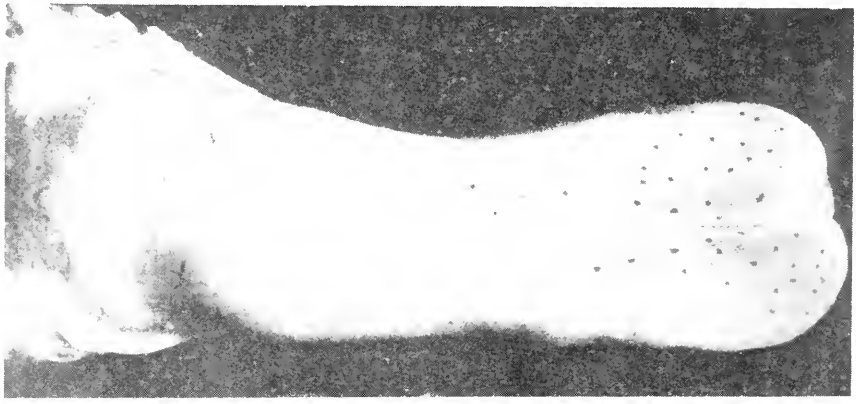

Figure 3. Surface view of tongue of rat after methylene blue injection showing distribution of fungiform papillae. After Fish, Malone and Richter (13).

portion of the lingual nerve. These taste fibers branch away from the lingual nerve to comprise the chorda tympani nerve, which then passes through the middle ear and joins the facial nerve. The posterior third of the tongue is innervated by the glossopharyngeal nerve. The vagus nerve may receive taste fibers from other portions of the oral cavity.

The end organs of taste are unusual in their close association with their nerve supply. Thus if one cuts the nerve, both the nerve and the taste buds it innervates degenerate. During the eventual regeneration of the nerve fibers, new taste buds are formed from the epithelial tissue of the tongue, so that ordinary epithelial tissue is transformed into specialized taste cells that are able to respond to dilute solutions of various kinds. How this transformation is accomplished is not known.

Papillae with their associated taste buds have been observed in the foetus. In the rabbit, Hermann has observed atrophy of these buds shortly before birth and then a complete reappearance of them by the sixth day (table 2).

Although it has been shown that the taste end organs are functionally of different types, no anatomical differences among the taste buds of the tongue have been observed. Recently, however,

Table 2. Papillae diameter

Age of rabbit	Foliate	Vallate	Taste buds
Foetus, 54-mm	0.045-mm	0.21	appear.
Foetus, 70-mm	.055	.39	complete.
Foetus, 95-mm	.07	.46	atrophied.
Newborn	.1	.49	rarely buds.
2 days old	.104	.54	appear.
3 days old	.11	.61	appear.
4 days old	.12	.68	
5 days old	.13	.82	
6 days old	.148	------	complete.
Adult	.2	------	

From Hermann (*16*).

the enzymes that are present in the taste buds and surrounding tissue have been studied *(6)*. Histochemical preparations have revealed that esterase, hexose diphosphatase, 5-nucleotidase, acid phosphatase, lipase, 3-nucleotidase, and ribonuclease are all localized in gustatory regions of the tongue. These enzymes are present not only in the taste buds, but some also are localized in neighboring tissue.

Methods of Study

Quantitative and objective data concerning the response of taste cells to stimuli have been obtained from the study of experimental animals using modern biological techniques. The method found most useful in the study of other sensory systems is the recording of the electrical nerve potentials that are generated in response to stimulation of the receptor. If the nerve message is recorded from the primary neuron that directly innervates the receptor, the analysis of the data is much easier than if the results are obtained from the higher centers, such as the brain.

The chorda tympani nerves innervate the taste buds of the anterior two-thirds of the tongue. Each taste nerve fiber is about 3 micra in diameter. If one electrically records from one such isolated fiber, information concerning the response of one or more taste cells within a single taste bud is obtained. A fine microelectrode inserted into a single taste cell will provide information from but one taste cell of the taste bud. On the other hand, the response of a population of receptors may be obtained by recording from the whole chorda tympani nerve and electronically integrating the total electrical activity (figs. 4 and 5). This latter method allows one to measure quantitatively and reproducibly the responses to thousands of taste stimulations in one animal over a

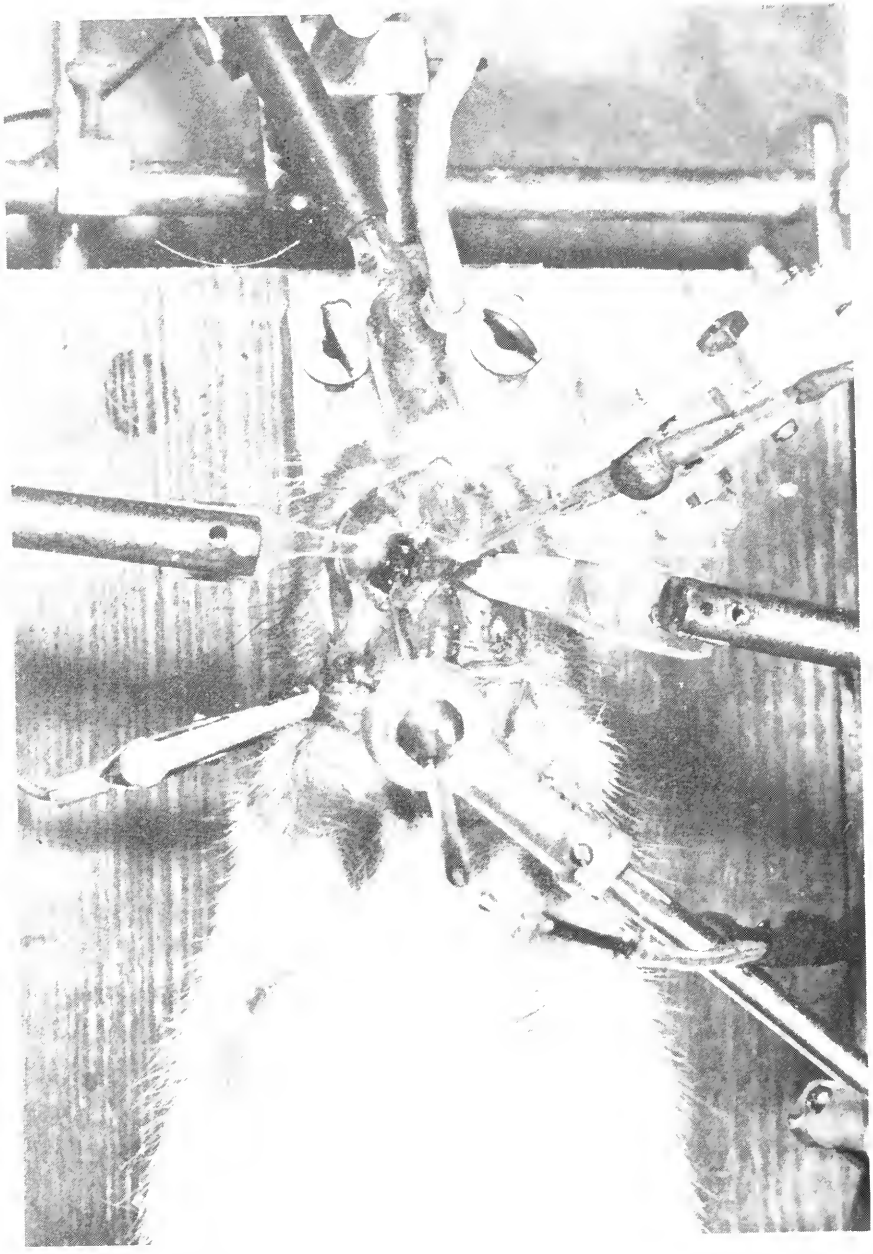

Figure 4. Ventral view of **rat taste** *preparation.*

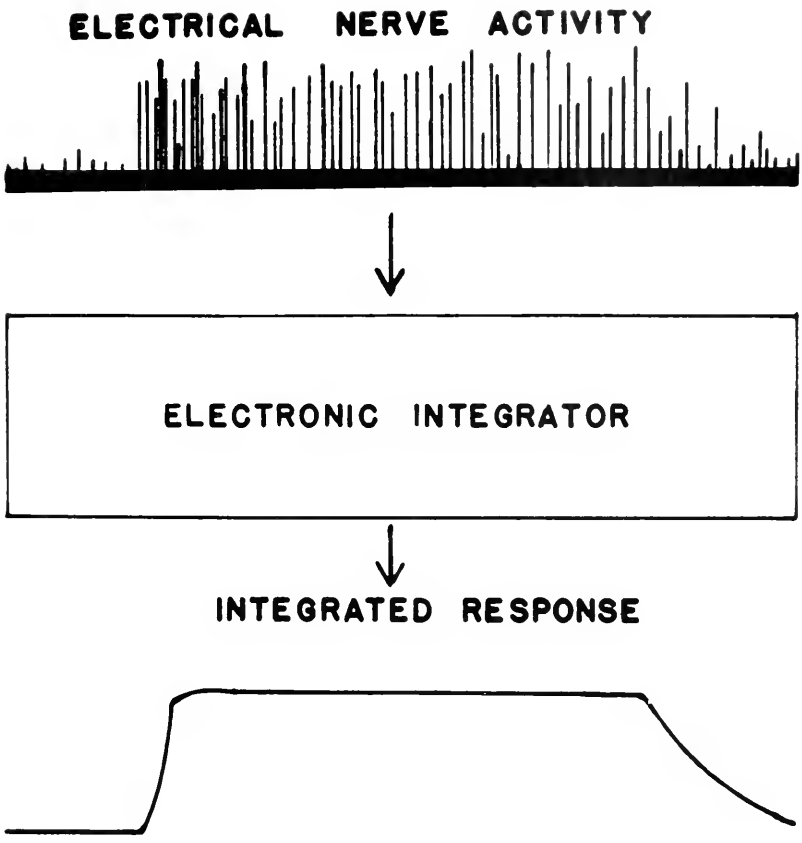

Figure 5. Schematic showing quantification of neural activity.

period of 12 to 18 hours. To observe changes with hormones, nerve regenerations, etc., objectively over a period of weeks or months, techniques are being developed to record nerve responses in the rabbit by means of permanent implanted electrodes.

The stimulus also must be quantified. This means that the time of application, the concentration of the applied chemical, the physico-chemical properties of the stimulus, the area of stimulation, the influences of saliva and temperature, and the previous history of the preparation all must be controlled. A flow chamber around the tongue eliminates salivary effects, and allows one to stimulate consistently a given area of the tongue (fig. 6).

The choice of an experimental animal depends upon the particular question asked. In general a mammal is chosen so that one may correlate the data with human behavioral information. Which mammal is selected depends upon the type of stimulus

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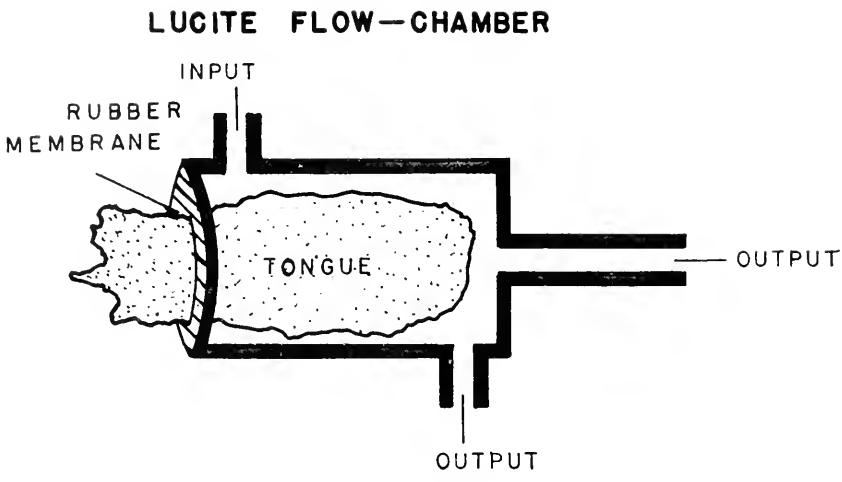

Figure 6. Flow-chamber for rat tongue.

one would like to study. For example, rats respond very well to salts and acids but not too well to sugars *(7)*. On the other hand, hamsters and guinea pigs readily respond to sugars (table 3).

Table 3. Relative magnitude of response

Chemical stimulus	Rat	Hamster	Guinea Pig	Dog	Cat	Rabbit
0.5 M NH_4Cl*				1.0	1.0	1.0
0.1 M NaCl	1.0	1.0	1.0			
0.01 M HCl	0.61	0.85	0.44	0.16	0.36	0.56
0.5 M Sucrose	0.21	0.75	0.62	0.27	0-0.20	0.52
20 mM Quinine–HCl	0.20	0.33	0.24	0.09	0.31	0.48

* 0.5 M NH_4Cl, rather than 0.1 M NaCl was used as a standard in those animals that did not respond well to NaCl. All responses were calculated as fractions of the standard response.

Experimental Results

Salts. How fast does a receptor respond to a chemical stimulus? This question may be answered by flowing 0.1 M NaCl over the tongue and measuring the time between this application and the moment the first electrical activity from the nerve is recorded. This latency is about 50 milliseconds or less. The question then arises, what is the contribution of the diffusion rate to this latency? This may be answered by calculating the time for diffusion of 0.1 M NaCl through a 10 micra layer of unstirred water to the receptor. Such a calculation shows that diffusion is ex-

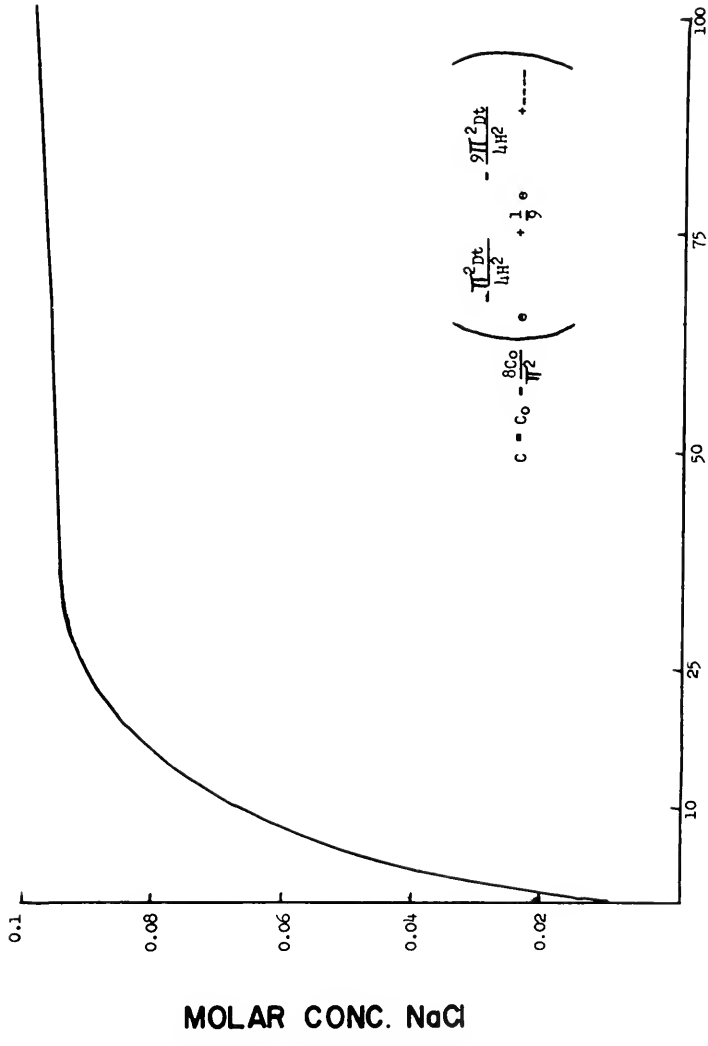

Figure 7. Diffusion of 0.1 M NaCl *through a 10 micra layer of water.*

tremely rapid over these small distances and that the time for diffusion does not contribute very much to the latency (fig. 7). It can be shown that a threshold concentration, 0.002 M NaCl, arrives at the receptor a few milliseconds after 0.1 M NaCl is applied to the tongue.

What differences in responses are observed with various salt stimulations? If equal molar concentrations of a given series of different chloride salts are applied to the tongue, one observes that the response is not the same for all salts (fig. 8). In order

Figure 8. Integrated electrical response of chorda tympani to various 0.1 M chloride salt solutions flowed over tongue of rat: NH_4Cl, LiCl, NaCl, KCl, NaCl, RbCl, CsCl, NaCl. *Test solutions are interspaced with water rinses. Time scale: 1 large division 20 sec.*

to utilize this information in a theory of taste mechanism, one must measure the response to various concentrations of these salts. If this is done, it is observed that the maximum response to one salt is not the same as the level of maximum response to another *(8)*. In a similar manner, one may study the anions and their effects upon the stimulation. If an homologous series of sodium organic salts is used as the stimulus, the resultant concentration curves indicate a small effect of the anion (fig. 9).

Enhancement with lithium citrate has been observed, i.e., if lithium citrate is applied to the tongue, the response to subsequent NaCl applications is increased (fig. 10). No such enhancement has been observed with various concentrations of sodium glutamate.

The electrical activity recorded from a taste nerve in response to salt stimulation decreases during the first 2 seconds, and then usually maintains a steady state when monovalent salts are used. The response to divalent salts slowly declines until complete adaptation occurs. It is of interest to note that if complete adaptation to $CaCl_2$ is obtained, a subsequent stimulation with NaCl will result in a normal NaCl response (fig. 11). Nevertheless, other

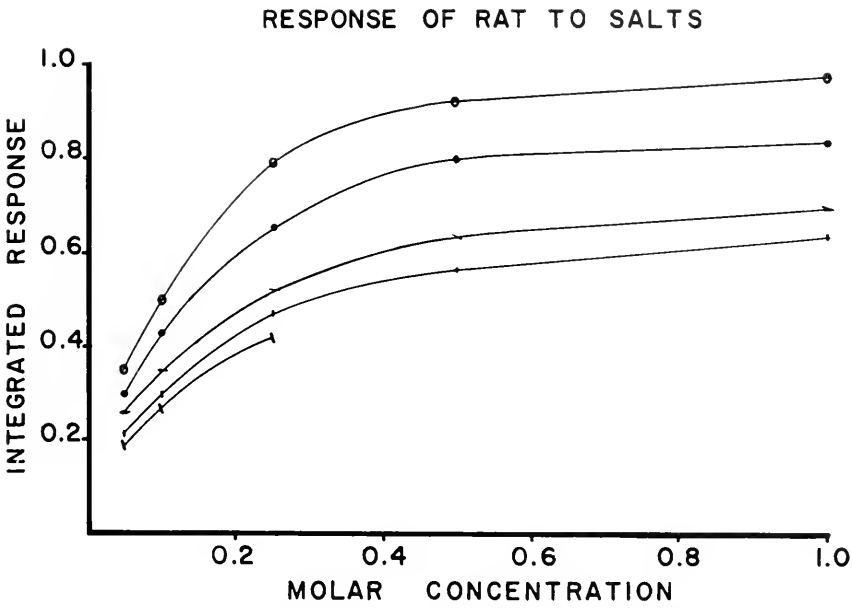

Figure 9. Magnitudes of responses of rat to sodium organic salts. Top to bottom: NaCl, Na *formate,* Na *acetate,* Na *propionate,* Na *butyrate.*

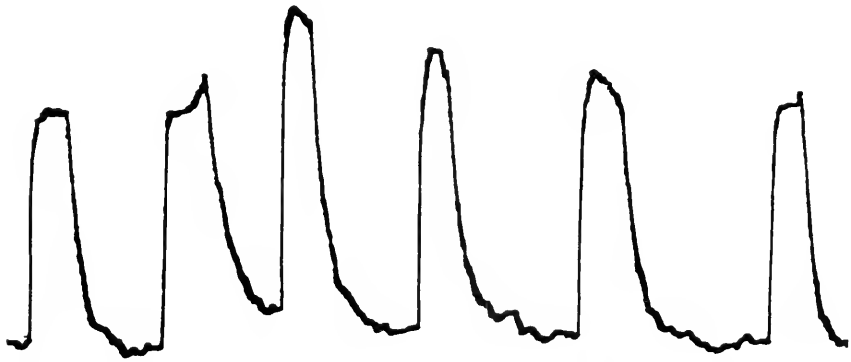

Figure 10. Integrated electrical response of chorda tympani of rat to 0.1 M NaCl, Li_3, *citrate,* NaCl, NaCl, NaCl, NaCl *interspaced with water rinses. Time scale: 20 sec.*

experiments indicate that NaCl and $CaCl_2$ actually stimulate the same cell. This means that cross-adaptation is not a good criterion for determining whether one is dealing with one type of receptor or more. Complete adaptation to NaCl reported by human subjects must, therefore, be explained by adaptive processes at higher levels of the central nervous system, and not at the periphery.

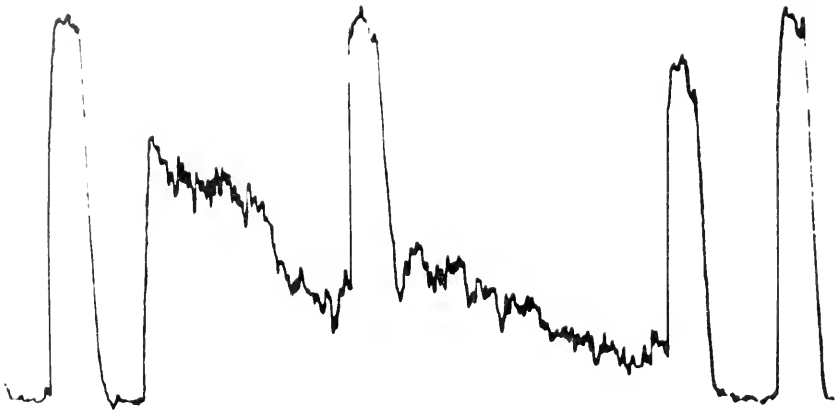

Figure 11. Effect of 0.1 M $CaCl_2$ *adaptation upon 0.1 M* NaCl *response. 0.1 M* NaCl *control was followed by continuous application of 0.1 M* $CaCl_2$ *with two 0.1 M* NaCl *responses before rinsing with water. The last response was to 0.1 M* NaCl. *Time scale: 20 seconds.*

Species differences. It is important to know what differences in taste responses there are from one species to another in order to see how general a theory is in its application. Furthermore, much of the physiological information has been obtained from experimental animals and not from man, although the interest of people in the food industry is in taste perception by humans. Clearly, it would be advantageous to know how much difference to expect when one compares the taste of the rat with that of man.

A number of different species of animals were utilized, and the same method of electronically recording the electrical nerve impulses was used *(7)*. No two species of animals were identical in their taste responses. However, some generalizations could be made. All the rodents responded very well to taste stimuli. The response to NaCl was much greater than to KCl at the same concentration. This was not true of the carnivores, which had low taste sensitivity, and the response to NaCl was always smaller than that to KCl of the same concentration. Furthermore, there were differences within any given order of animals. For example, the rat responded poorly to sugar solutions, whereas the hamster and guinea pig responded very well. Although about 12 different species have been studied in this laboratory, no correlation of response to various solutions and the eating habits of the animal has been made. However, correlation can be made between the responsiveness to NaCl and KCl, and the concentration of these ions in the interior of the red cell. This correlation is excellent, as shown in table 4, but it is difficult to see a causal relationship.

Table 4. Correlation between responsiveness to sodium chloride and potassium chloride

	Red cell content $\frac{Na}{K}$	Relative taste response $\frac{Na}{K}$
Carnivores:		
Raccoon	19.3	0.75
Cat	17.0	0.67
Dog	12.0	0.43
Average	16.1	0.62
Rodents:		
Rat	0.12	2.0
Hamster	0.11	2.8
Guinea pig	0.14	2.6
Average	0.12	2.5
Marsupials:		
Opossum		0.45

Zotterman *(5)* found that the frog exhibits a taste phenomenon called water taste, i.e., the electrical nerve activity may be greater to water than to low concentrations of NaCl. This phenomenon also has been discovered in some mammals, particularly the rabbit, in which water flowing over the tongue produces a rather strong response *(7)*. If low concentrations of NaCl are flowed over the tongue, the response declines. Zotterman *(27)* suggests that water taste may be important in the detection of low concentrations of taste substances by animals such as the rabbit. This concept cannot be applied to an animal such as the rat because water taste is not present, and the animal responds to very low concentrations of salts.

Acids. Most sour compounds have in common the hydrogen ion. However, it has been shown by many workers that there is not a direct relationship between the magnitude of the hydrogen ion concentration and the magnitude of the sour response itself. Taylor *(24)* concluded that in man the magnitude of the sour response depends upon the pH of the interior of the taste cell. To obtain a given pH depends not only upon providing a particular hydrogen ion concentration, but upon the ability of a particular acid to penetrate the cell. In order to verify or reject Taylor's hypothesis, we have studied the response of the rat to each of a group of acids flowed over the surface of the tongue and isolated from salivary influences. One does not obtain the same magnitude of response with various acids of equal pH (fig. 12), and one does not obtain the same magnitude of response with equal molarities of acids. Likewise equal responses of a given number of acids correspond to different concentrations (table 5). When the

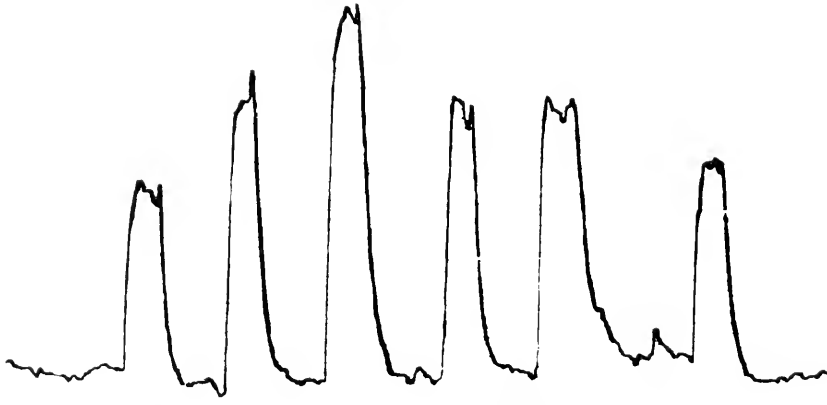

Figure 12. Integrated response of rat to hydrochloric, citric, formic, oxalic, acetic and hydrochloric acids at pH = 2.5. Time scale: large unit = 20 seconds.

Table 5. Response equal to that of 0.0050 HCl

Acid	Molar Conc.	Acid	Molar Conc.
Sulfuric	0.0022	Succinic	0.0100
Oxalic	0.0033	Monochloroacetic	0.0104
Hydrochloric	0.0050	Glutaric	0.0110
Citric	0.0055	Formic	0.0116
Tartaric	0.0059	Adipic	0.0140
Nitric	0.0059	Glycolic	0.0150
Maleic	0.0064	Lactic	0.0156
Dichloroacetic	0.0090	Mandelic	0.0250
Malic	0.0100	Butyric	0.1500

magnitude of response is plotted versus concentration it is observed that the saturation level, or the maximum response obtainable, differs from one acid to another. This finding would appear to reject the hypothesis of Taylor that equal pH's within the interior of the cell correspond to equal magnitude of response. According to Taylor's theory, one should be able to obtain a given pH by merely adding more acid to the exterior of the receptor, so that all acids reach the same saturation level, but at different concentrations.

Sugars. Early work by Zotterman *(28)* resulted in the concept that there are no sugar fibers in the cat. Subsequent work by Pfaffmann confirmed this idea. Later both Pfaffmann *(20)* and Beidler *(7)* studied a number of different species and it was found that the cat indeed does have sugar fibers but very few of them. On the other hand, animals such as the hamster and the guinea pig have very good sugar responses (table 6). Sucrose always

Table 6. Sugar responses of hamster and guinea pig

Substance	$-\Delta F$ kcal
HCl	2.42
Sodium chloride	1.37
formate	1.32
acetate	1.29
propionate	1.22
butyrate	1.23
Ammonium chloride	1.10
formate	0.91
acetate	0.74
Lithium chloride	1.34
acetate	1.24
Magnesium chloride	1.23
formate	1.23

stimulates well in these animals, whereas glucose stimulates to a lesser degree. Substances like lactose stimulate poorly. It is interesting to note that sodium saccharine does not stimulate the sugar receptors very well but does stimulate the salt receptors at higher concentrations, as would be expected.

Bitter. Very few experiments have been performed with the glossopharyngeal taste nerve, which innervates the back third of the tongue. This is unfortunate, since this region responds very well to bitter substances. However, the preparation is difficult, and therefore very little objective data exists with bitter substances.

The classical 4-taste qualities. The concept that there exists 4-taste qualities has remained in the taste literature for many years. There are many good reasons for this, since many experiments have shown this to be a good hypothesis. What is the receptor basis for the 4-taste qualities? The answer to this can be found in single fiber studies as well as single taste cell studies.

Single fiber data. Pfaffmann *(20)* showed with single fiber studies in the cat that there exist fibers that respond to several of the taste qualities. That is, one fiber may respond to both acids and salts, another to acids, salts and bitters, and so forth. Both Pfaffmann *(21)* and Fishman *(14)* made a more extensive study using rats and hamsters. They concluded that each fiber differs in its sensitivity to the 4-taste qualities. Some fibers respond to 1, 2, 3, or 4 of the qualities. Similarly, the sensitivity of a single fiber to NaCl varies from fiber to fiber. If one observes the magnitude of response to a given series of chloride salts, one finds that there is also a variation in the response series from one fiber to another.

1 0.1 M NaCl
2 0.5 M Sucrose
3 0.02 M Quinine hydrochloride
4 0.05 M HCl

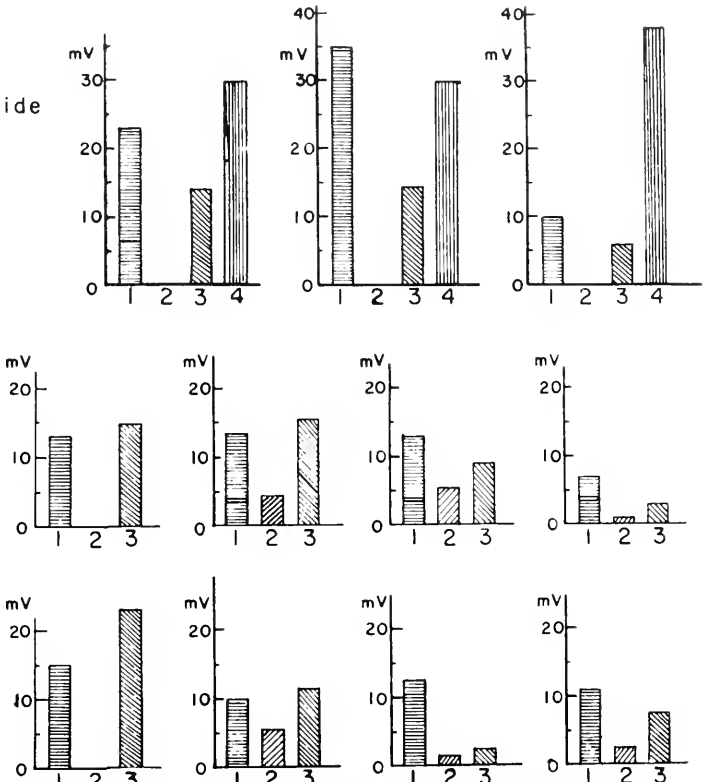

Figure 13. Magnitude of change in resting potential as recorded with microelectrode in taste cell.

Unfortunately, the single fiber data described above does not answer the question unequivocally, since one taste fiber goes to more than one taste cell within a single taste bud. Does one taste cell only respond to one taste quality, or is there also a mixture at the taste cell level? To answer this question, one must record from the single taste cell.

Taste cell studies with microelectrodes. Dr. Kimura in our laboratory placed very fine microelectrodes into the single taste cells of the single taste bud of the rat. He then studied the response of these cells electrophysiologically as various solutions were flowed over the surface of the tongue. His results were similar to the single fiber data (figs. 13 and 14). One must conclude that a single taste cell can respond to several of the 4-taste qualities, and great variations in sensitivity occur from one taste cell to the next.

A taste theory and its verification. A number of facts have been stated in this paper as a result of many experiments. How can one organize this material into a useful theory of taste mechanism? Let us assume that the salt cation is bound to a receptor molecule. The reaction then would follow the general reaction:

$$A + P \rightleftharpoons AP$$

A represents the cation, P represents the unbound sites, and AP the bound sites. Let C equal the concentration of cations in solution, n the total number of binding sites available, and Z the number of sites filled at any stimulus concentration, C. Therefore, $n - Z$ is the number of sites unfilled. The equilibrium constant, K, is defined as:

$$K = \frac{Z}{C(n - Z)}$$

The response of the receptors should be proportional to the number of sites filled or:

$$R = aZ$$

where a is a constant of proportionality. The maximum response, R_s, occurs when all the available sites are filled or:

$$R_s = a\,n$$

Substituting this information into the original equation containing the equilibrium constant, one obtains a taste equation:

$$\frac{C}{R} = \frac{C}{R_s} + \frac{1}{KR_s}$$

This is an equation of a straight line where the ordinate is $\frac{C}{R}$ and the abscissa is C. The slope is equal to $\frac{1}{R_s}$ and the intercept is equal to $\frac{1}{KR_s}$. This equation is similar to Langmiur's

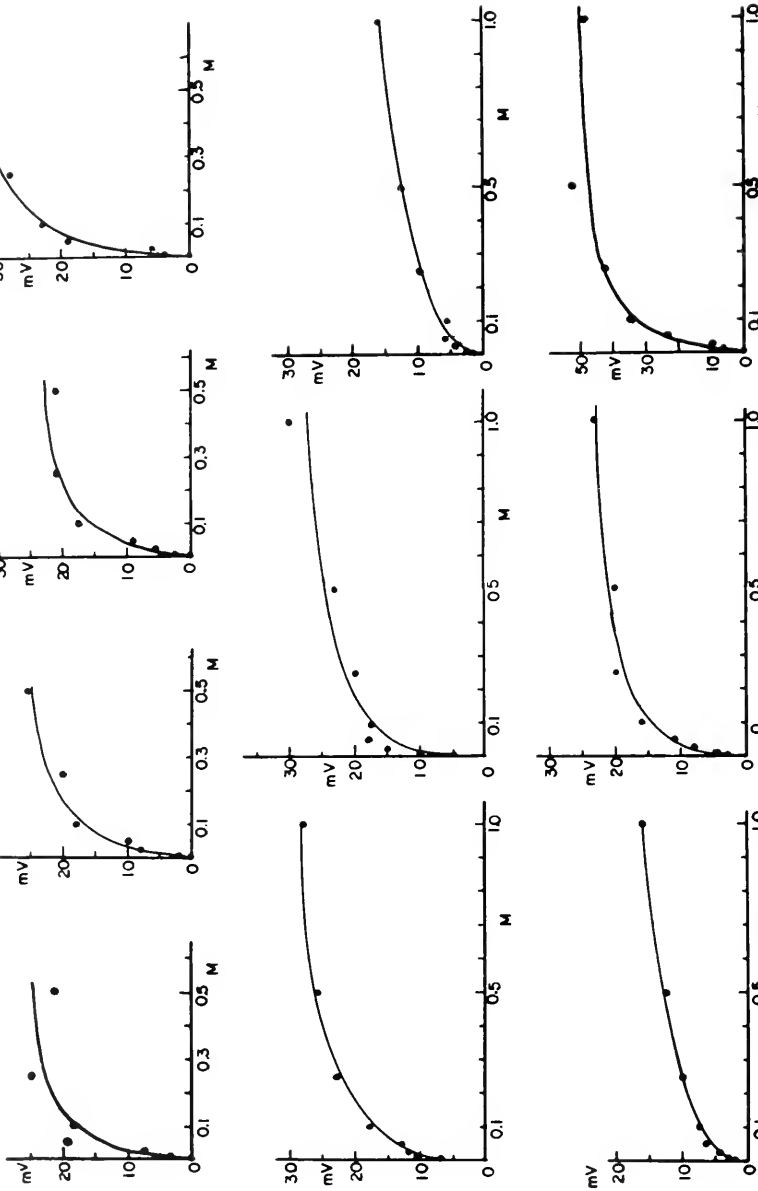

Figure 14. Magnitude of change in resting potential of taste cell in response to various concentrations of NaCl. *Each graph represents a different taste cell.*

adsorption isotherm. It is also similar to the equation derived in considering the adsorption of cations by proteins. One may test the applicability of this equation by plotting the experimental values of $\frac{C}{R}$ against C. A plot of this data for various organic salts shows that a straight line results (fig. 15). This

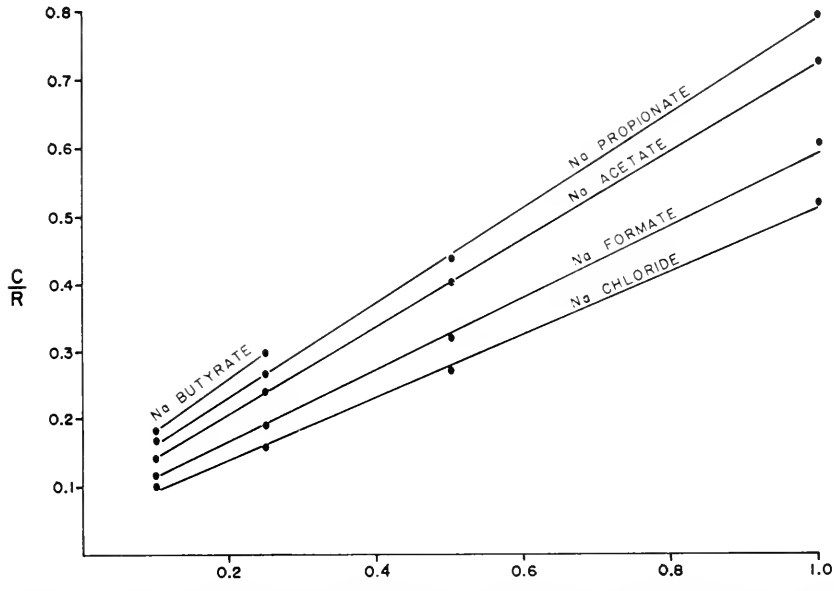

Figure 15. Ratio of molar concentration of stimulus and magnitude of integrated response of taste receptors is plotted against molar concentration of stimulus.

means that the taste equation is sufficient to describe the experimental taste data.

It can be shown that a state of equilibrium exists between the receptors and the salts 2 seconds after stimulation. Therefore, the change in free energy of the reaction may be obtained:

$$\triangle F = -RT \ln K$$

R is the universal gas constant, T is the absolute temperature, and K is the equilibrium constant. The calculated change in free energy is very small as shown in table 6. This means that the presence of enzymatic reactions in the first step of taste stimulation is very doubtful, but that very weak forces exist between the cations and the receptor molecules. The nature of the receptor molecule that is binding the cation can be elucidated by varying the pH of the taste solution and observing the change in

response. No change occurs between the pH of 3 and 11 *(9)*. Therefore, one may assume that carboxyl groups are not binding the cations, but phosphate or similar groups may be involved. Thus a natural polyelectrolyte on the surface of the receptor could easily bind these cations. Temperature studies give more evidence for such a concept. One may vary the temperature 10° around room temperature without observing any change in the response to salts. This means most of the change of free energy is actually a change in entropy. One may conclude that either the hydration of the ion changes as it is bound to the receptor molecule or else the receptor molecule changes its spatial configuration.

Since it has been shown that the force binding the taste substance to the receptor is very small, one would expect that small changes in the spatial configuration of the receptor molecule would change the binding properties. Therefore, one should expect differences from one receptor to another in the binding action of these salts. One also should expect differences from one species of animal to another in a manner similar to the binding differences observed from one synthetic ion exchange to another. The reversal of the sodium-potassium ratio as found to exist between rodents and carnivores can easily be explained with this hypothesis. The binding of these ions depends very much upon the electrical atmosphere surrounding the binding site *(18)*. Actually the sodium may be in a hydrated state when it is bound. On the other hand, the receptor molecule may be such that the sodium will be in a partially hydrated or nonhydrated state when it is bound. In the latter case, sodium should be bound stronger than potassium, as is the case with rodents.

The anion as well as the cation is bound to the natural polyelectrolyte. This accounts for the lack of a direct relation between the hydrogen ion concentration of an acid and its ability to evoke a response from the taste receptor. Similarly, since the adsorption of a molecule depends upon the peculiar spatial configuration of the receptor molecule as well as its side groups, which may vary from one individual to another, genetic taste differences are not unexpected.

The large number of different substances that can stimulate the taste receptors over a large range of concentrations suggest the substances need not enter the cell. One actually may place 0.1 M NaCN on the tongue of the rat and obtain a response similar to that from NaCl. No irreversible effects are observed. If the cyanide entered the cell in any appreciable concentration, obviously it would have an effect on the cellular respiration and func-

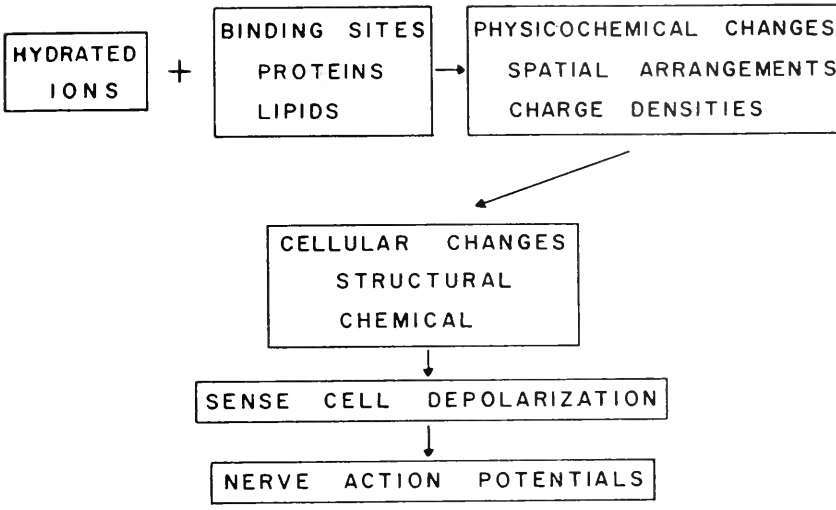

Figure 16. Schematic of proposed mechanism of taste stimulation.

tion. Figure 16 schematically shows the processes of taste stimulation with electrolytes.

The above adsorption theory of taste was developed from considerations of the electrolyte stimulation of the taste receptors. Studies of the responses to sugars indicate that the forces of attachment to the receptor surface are also weak. Van der Waals forces probably play a great role in such stimulation. Since the nonelectrolytes so far studied are much larger in size than the electrolytes, there is a greater emphasis upon the spatial configuration. This is particularly true in the chemical specificity in genetical differences of taste sensitivity as shown by Harris and Kalmus *(15)*.

Taste theory as applied to human studies. Up to this point in the discussion, most of the data presented have been obtained from experimental animals. Naturally one would like to correlate the information with the human. If the fundamental taste equation is a general one, it should apply to human data. However, it is difficult to obtain quantitative information concerning supra-threshold concentrations of taste stimuli. There is one approach, however, that can be used and that is the study of the just noticeable differences or JND's.

The fundamental taste equation may be rearranged and solved for the response, R.

$$R = \frac{C K R_s}{C K + 1}$$

Then, $\triangle R$, the change in response, between two different concentrations, may be calculated as:

$$\triangle R = R_A - R_B = \frac{C_A K R_s}{C_A K + 1} - \frac{C_B K R_s}{C_B K + 1}$$

The change in neural activity for a given JND should be constant, i.e., equal increments of neural activity correspond to equal magnitudes of responses. This assumption is very prevalent in the psycho-physical literature. Let $\triangle R = M$, a constant. Solve for $\triangle C$ which is equal to $C_A - C_B$. In this manner, one obtains:

$$\triangle C = \frac{M(1 + 2KC_B + K^2C_B^2)}{K(R_s - KMC_B - M)}$$

This equation can be applied to threshold values by allowing $C_B = 0$. Then, since $R_s >> M$, $\triangle C$ is equal to $\frac{M}{KR_s}$. This means that the threshold value of a substance depends upon the equilibrium constant of the reaction and the maximum response obtainable with that particular substance.

Since K and R_s both vary from one substance to another and from one species to another, the thresholds also vary. The value of M or the minimum change in neural activity that is discriminated by an animal is dependent upon the physiological and psychological conditions under which the animal is operating. Thus it is possible to change the taste preference thresholds of an experimental animal by using proper methods of conditioned responses.

In order to determine whether the above equation now can be applied to human data, consecutive JND's of human observers were determined with NaCl solutions. The threshold value was

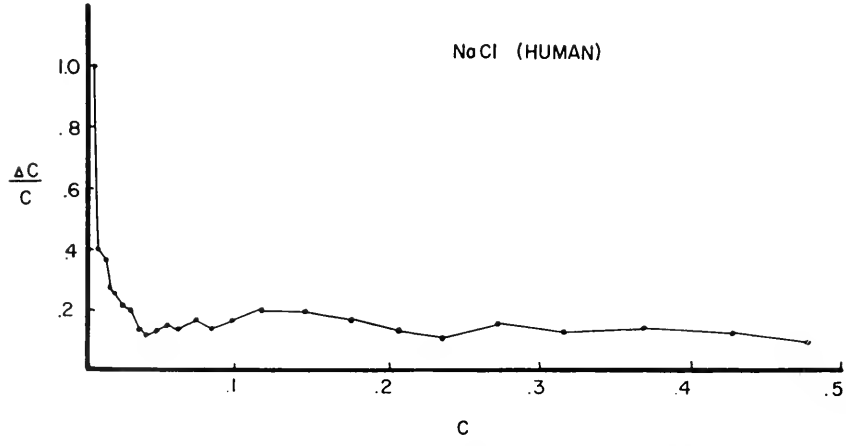

Figure 17. Plot of ratio of human JND values for NaCl *and* NaCl *concentration against* NaCl *concentration for consecutives JND's. All values determined on same subject.*

found and then the next JND was found, using threshold as the lower concentration, etc. (fig. 17). In this manner the total number of JND's could be obtained.

If equal neural activity represents equal responses, then each JND indicates the change in concentration necessary to elicit a given constant increment of neural activity, M, as indicated above. Since consecutive JND's were measured from threshold to a given concentration, each JND may be assigned a constant arbitrary value for the magnitude of response. In this way a plot of $\frac{C}{R}$ versus C may be obtained for humans and the equilibrium constant, saturation level, etc. may be computed. Since the plot approximates a straight line over a concentration range of 100–1,000 fold, the human data can be expressed by the derived taste equation.

One may conclude from these quantitative determinations that one should not base a theory of taste merely upon threshold data, because the threshold depends upon the product of two variables, the equilibrium constant and the maximum response. A threshold series for a group of taste substances is not indicative of the series obtained at higher concentrations. In other words, the threshold series of taste stimuli is not directly related to the strength of binding between the taste stimuli and the receptor. This emphasizes the importance of obtaining quantitative data concerning the response to above threshold concentrations of stimuli. Unfortunately, this cannot be easily accomplished with human observers, and therefore, one must rely upon experimental animals.

Olfaction

Introduction

Theories concerning the mechanism of olfactory receptor stimulation appear periodically. Most of these theories suffer from the lack of sufficient information concerning the response of the olfactory receptors. Usually human olfactory thresholds and cross-adaptation data are utilized in the formulation of a theory. Threshold data suffers from the same difficulties that were outlined in the previous discussion of taste. Likewise, it is usually assumed from cross-adaptation experiments that only one receptor responds to two substances if they cross adapt to each other. This involves an assumption that was shown not to be necessarily correct for taste stimuli. Furthermore, it is not

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known whether the adaptation studied is a peripheral or a central phenomenon. Adrian has proposed that olfactory adaptation occurs at the level of the olfactory bulb.

The present discussion will concern itself with objective and quantitative information obtained from the response of the olfactory receptors, and will not consider theories of olfaction in detail.

The Morphology of the Olfactory Structures

Olfactory receptors are limited to a small area of the nose on portions of the turbinates and the nasal septum. Trigeminal fibers which also respond to odors, supply the nose throughout.

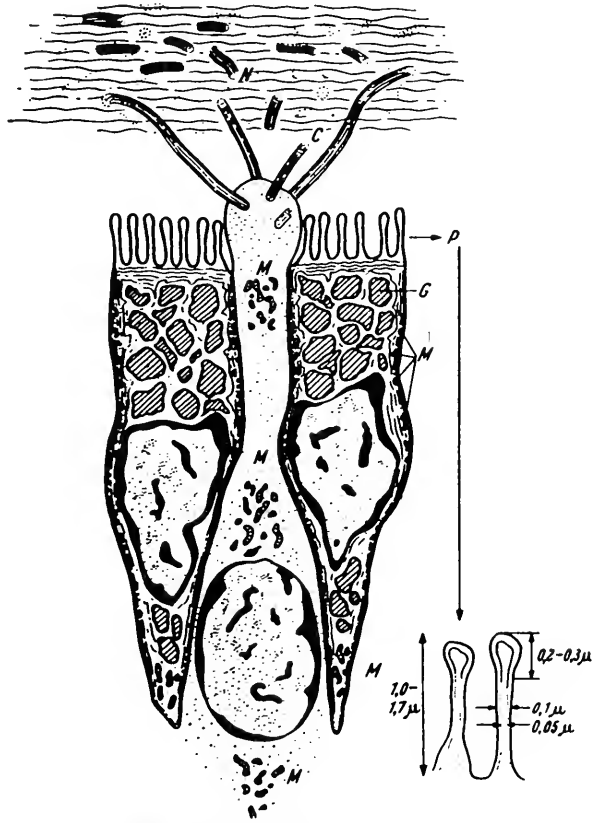


Figure 18. Schematic drawing showing the structure of the olfactory epithelial surface in the frog as determined by electron microscopy. M, mitochondria; C, olfactory cilia; N, network on epithelial surface; G, granules in sustentacular cell; P, small cytoplasmic protrusions on sustentacular cell. After Boom (11).

Olfactory cells are contained in a yellow pigmented area of about one square inch. These cells are bi-polar, and the proximal end is a fine nerve fiber about 0.2 micron diameter extending from the cell body to the olfactory bulb (fig. 18). Distally, the olfactory cell terminates in a number of fine hair-like structures that intermingle in the mucous layer covering the olfactory area. It is not definitely known whether these hairs penetrate the mucous and therefore enter the gaseous environment of the nasal cavity. However, Hopkins has studied olfactory hairs in the frog and has shown that many of them pass to the surface of the mucous layer and then turn to lie parallel to the surface. If this be true in mammals, then it is possible that the molecules of odor need not penetrate the aqueous layer in order to stimulate the hairs. The hairs themselves may have a length of about 200 micra. Although the olfactory cells make up a large portion of the olfactory area, there are also supporting cells and numerous Bowman glands. The mucous above the olfactory area moves by means of numerous cilia.

Numbers of the small, fine olfactory nerve fibers come together as fasciculi which then join to form the olfactory nerves and penetrate the cribriform plate and enter the olfactory bulb. The small size of the olfactory nerve fibers and the presence of the bony cribiform plate have made the recording of electrical activity from the olfactory nerves very difficult. Each receptor cell has its own nerve fiber that goes directly to the olfactory bulb.

The olfactory area of the rabbit contains about 100,000,000 receptors *(4)*. Electron microscope studies indicate 6–12 so-called olfactory hairs per cell *(11)*. These structures are about 0.2 micron in diameter and 150 micra long. If it is assumed that these olfactory projections are the structures that respond to odors, then there exists a surface area in the rabbit of 540–1,080 cm^2 to which the odorous molecules may attach and thereby initiate the olfactory response. In other words, the rabbit has about a billion olfactory hairs whose surface area is the same order of magnitude as the total skin surface of a young rabbit!

It is interesting to compare these values with those calculated for the taste receptors. Such a comparison reveals that the olfactory surface area is about a million times greater than the gustatory. Of what functional significance is this? Pfaffmann *(22)* denervated the tongue of rats and showed that a large reduction in the number of taste buds did not result in a proportionately large effect on taste discrimination. Thus the keenness of the chemical senses of a given animal is not directly related to the number of sensory elements. It is suggested that the large

surface area of the olfactory receptors is necessary to discriminate a large number of different odors.

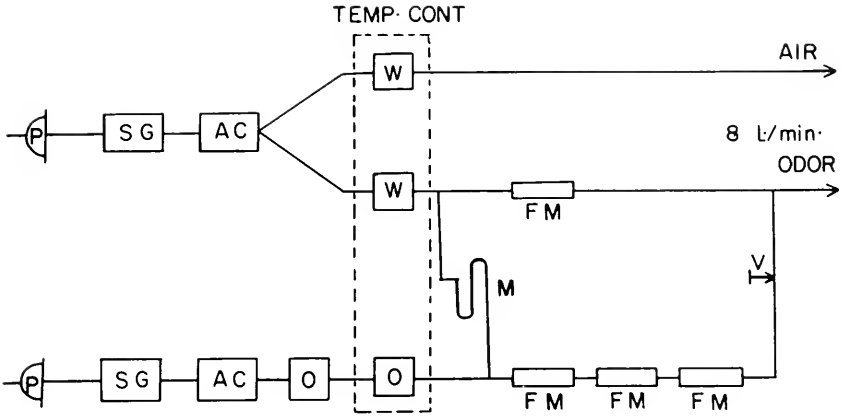
The bare endings of the trigeminal nerve fibers are found throughout the nasal area including the olfactory area. Since the nerve endings do not enter the mucous layer, all stimulating odors must pass through the mucous layer in order to excite the trigeminal nerve endings.

An odor entering the nares must pass a large moist surface area where some of the odor is adsorbed before it enters the olfactory region. Because there are numerous air passages through the nose, only a portion of the odor actually passes over the olfactory area.

Methods of Study

At the present time there exist only three good methods of obtaining objective and quantitative information from the olfactory area of an experimental animal. First, one may record from small electrodes thrust into the olfactory bulb and thereby obtain indirect information about the response of olfactory cells. Unfortunately, the response of the olfactory bulb depends upon the level of anaesthesia, and the records are not from the olfactory fibers themselves, but from the mitral cells *(2)*. Secondly, the dc potential across the olfactory tissue of an experimental animal may be recorded while an odor is flowed over the tissue. This technique has so far been applied successfully only to the olfactory tissue of the frog *(19)*. Thirdly, direct information about receptor responses may be obtained by recording the electrical activity of the olfactory nerve fibers *(10)*. At the present time, it is only possible to record from a number of these fibers simultaneously, and not from single fibers. This method has the advantage, however, of offering quantitative information. It is this latter approach that has been utilized by Don Tucker of our laboratory in the experiments to be described in this paper.

Quantification of the stimulus as it is presented to the nares of the animal is obtained by the use of an olfactometer and breathing chamber (figs. 19 and 20). The olfactometer cleans the room air and then saturates it with the vapor of the given odor. This odor is then mixed in a quantitative manner with purified room air by means of flow meters. The known concentration of odor is then passed into a breathing chamber. This chamber allows the animal to only breathe the odor and not air, when the odor is applied.



OLFACTOMETER

Figure 19. Diagram showing construction of olfactometer. P, pump; S G, silico gel; A C, activated carbon; W, water; O, odor; M, manometer; F M, flow meter; V, valve.

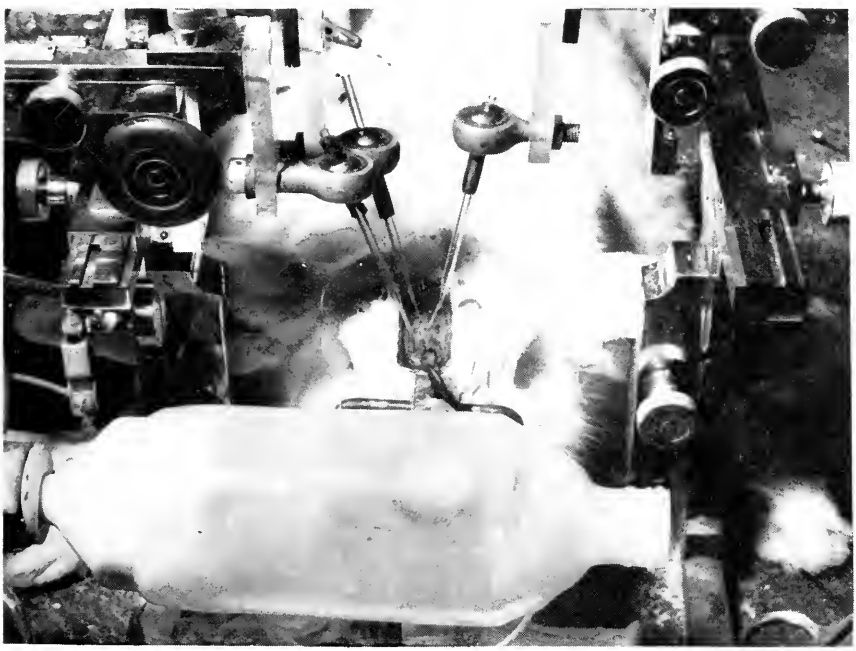


Figure 20. Rabbit olfactory preparation showing nerve recording equipment and breathing chamber.

It is possible to record directly from small trigeminal nerve bundles, and obtain a quantitative measure of the response of a number of trigeminal fibers to odors. This can be done either in a living animal, or in excised olfactory tissue (figs. 21 and 22).

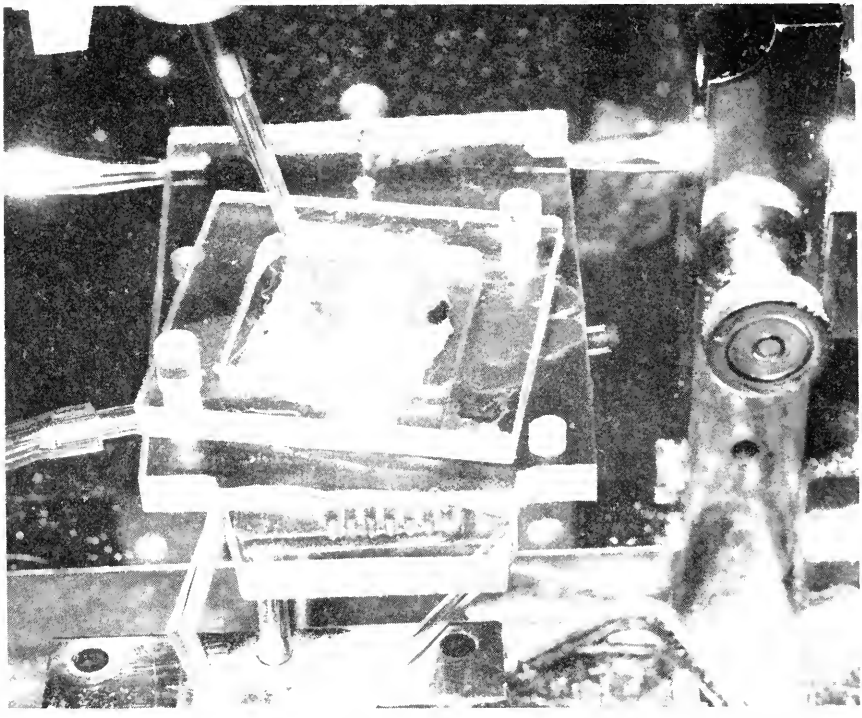


Figure 21. Opossum in vitro *preparation showing olfactory tissue including trigeminal nerves.*

Experimental Results

The olfactory cells are active during odor inspiration and quiet during expiration (fig. 23). The magnitude of this activity depends mainly on the type of odor and the concentration. At low concentrations of a given odor the same magnitude of response is observed during each inspiration. The response to moderate concentrations of an odor is greatest during the first inspiration of the odor and then declines to a steady level during succeeding inspirations. At very high concentrations this decline may continue to zero (complete adaptation). It is interesting to note that with the low concentration of odors no adaptation is seen from one inspiration to the next even though we know that complete adaptation does occur in human at low odor concentrations.

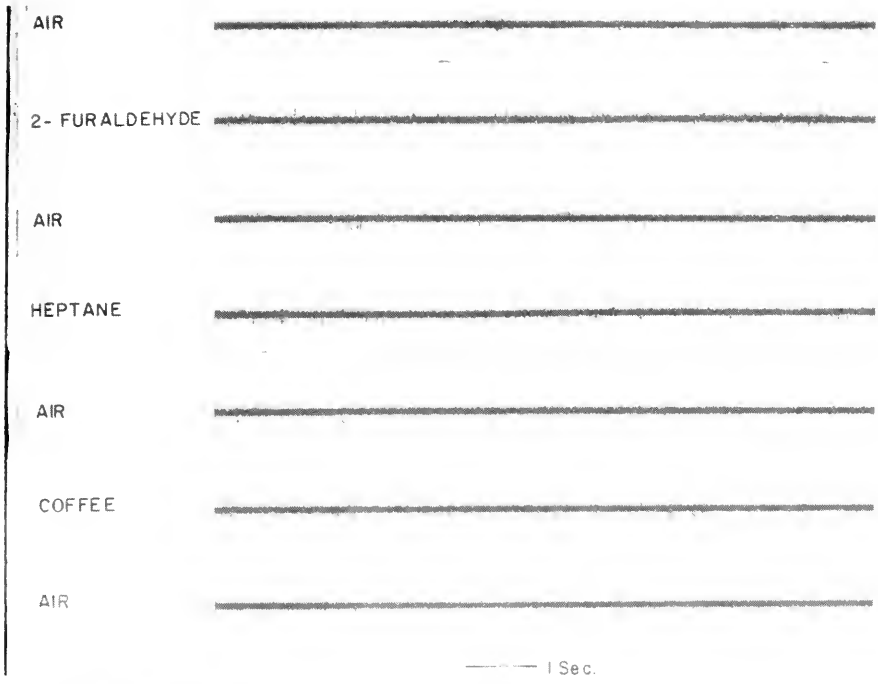


Figure 22. Trigeminal few fiber response to odors. Opossum in vitro *preparation.*

Therefore, one concludes that this type of adaptation must be found centrally as has been suggested by Adrian.

A quantitative measure of the response to various concentrations of odors is obtained by integrating the electrical activity. The threshold value for amyl acetate found in this manner is less than a half micromolar concentration (fig. 24). This figure agrees well with psycho-physical measurements of human threshold.

The trigeminal nerve fibers are stimulated by slightly higher concentrations of amyl acetate as well as by all other odors in concentrations not obnoxious to man. This leads one to suspect that the trigeminal sensory system is more important than formerly realized. The trigeminal nerve fibers do not respond as fast as the olfactory cells, but when they do respond the response increases in magnitude with time.

It is interesting to note that if high concentrations of odors are used on experimental animals, the dimensions of the nares may change, and therefore the amount of odor entering the nose may change. Also, the rate of breathing and therefore the flow rate to the olfactory area may change. These and other changes

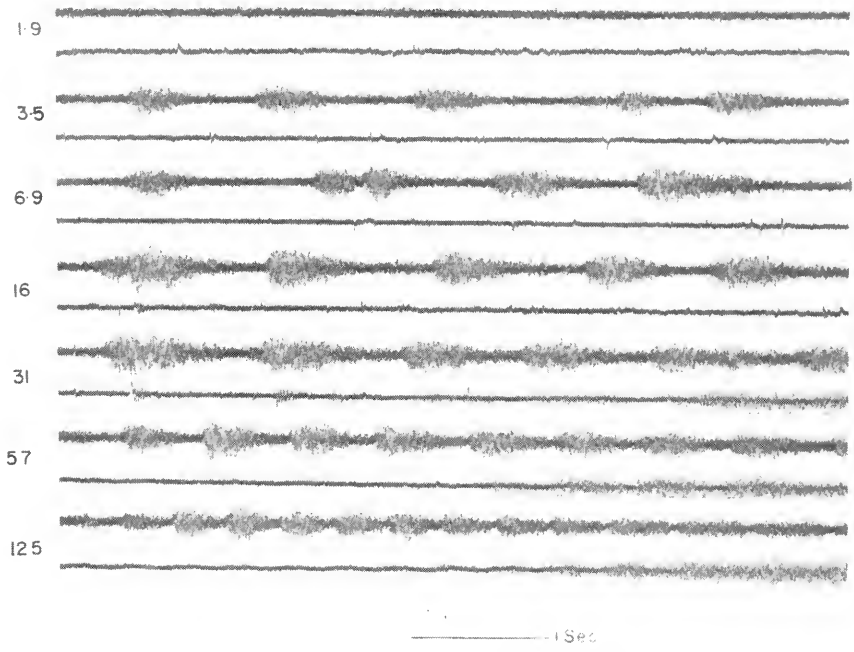


Figure 23. Olfactory and trigeminal nerve responses to various concentrations of amyl acetate inhaled by rabbit. Top trace of each pair is olfactory and bottom trace is trigeminal Micromolar concentrations, top to bottom: 1.9, 3.5, 6.9, 16, 31, 57, 125. Time scale: 1 second.

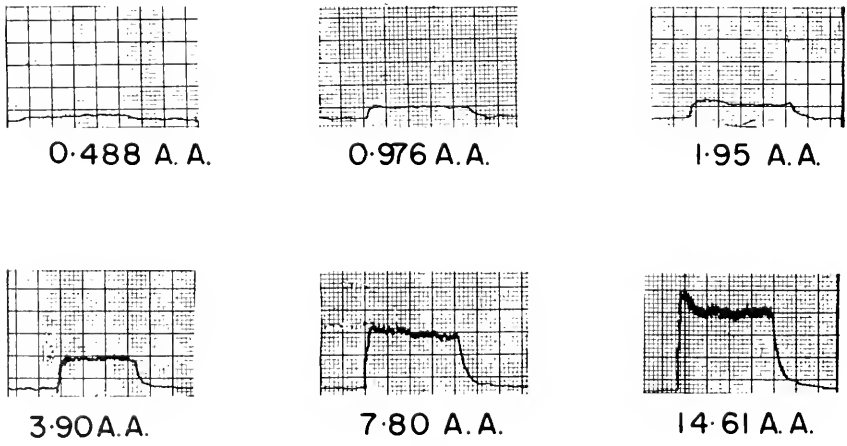


Figure 24. Integrated electrical activity of rabbit olfactory nerves in response to amyl acetate. Micromolar concentrations. Time scale: 1 large unit = 20 seconds.

within the nose require careful controls when working at higher concentrations.

In order better to understand the mechanism of stimulation it is necessary to apply a constant stimulus. Normal breathing is a periodic stimulus, and the response is difficult to analyze. The trachea may be cannulated and odors pulled through the nares by means of a syringe applied to the trachea. In the rabbit the magnitude of response depends upon the flow rate up to about one liter per minute (fig. 25). If the odor enters the nares for

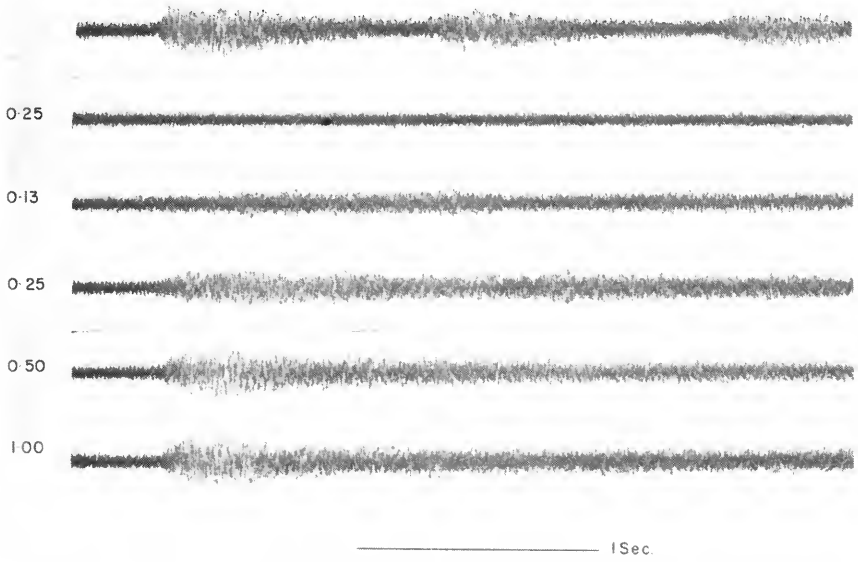


Figure 25. Olfactory nerve activity of rabbit in response to various flow rates of amyl acetate at constant concentration. First record is activity during normal breathing of odor. Following records were obtained by pulling odor through nares at constant rate with suction applied to the cannulated trachea. Second record is response to purified air and remaining records to amyl acetate. Values of flow rates in l/min.

many seconds, and then is stopped and then started again, a response during the stoppage is not observed even though no air entered the system during the entire experiment (fig. 26). This means that either the non-olfactory tissue of the nose adsorbs the odor so that as soon as the application of the odor to the nares stops the concentration in the olfactory area drops to zero, or else the response of the olfactory cell itself is dependent upon flow rate. It is obvious that the theory of mechanism of stimulation would depend greatly upon which of these two possibilities actually is correct.

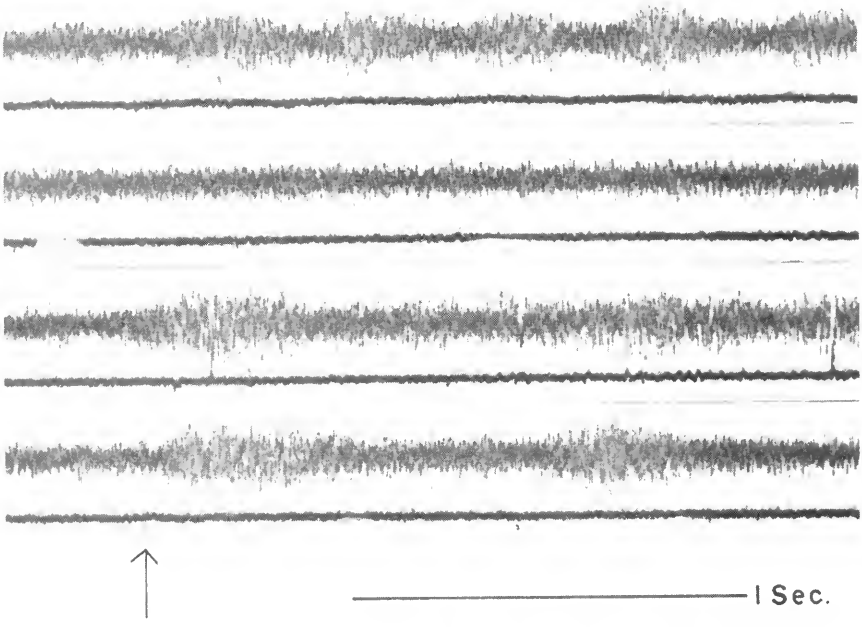


Figure 26. Electrical activity of rabbit olfactory (top trace) and trigeminal (bottom trace) nerves in response to amyl acetate of constant concentration. First record: normal breathing of amyl acetate. Second: purified air pulled through nares by way of cannulated trachea. Third: amyl acetate pulled through nares at constant rate. Fourth: amyl acetate pulled through nares in periodic bursts. No flow of odor between bursts. Arrow denotes application of stimulus.

Olfactory waves. Adrian *(2)* has shown that one can record olfactory waves from the olfactory bulb. That is, instead of recording electrical impulses similar to those observed from olfactory and trigeminal nerves, the activity occurs in waves of 30 to 60 cycles per second. The magnitude of these waves depends upon the magnitude of the stimulus, but the frequency remains constant (fig. 27).

Qualities of odor. How does an animal differentiate the qualities of odors? Little information bearing upon this question has been obtained from the techniques outlined above. Adrian *(3)* and others have found, however, that the olfactory bulb responds better in one area than in another to certain qualities of odor. It is possible that one area of the olfactory tissue responds to one type of odor and another area to another type of odor. This would then be the basis for odor discrimination. Up to the present time no one has been able to record from single olfactory nerve fibers. Therefore, we do not know how the different re-

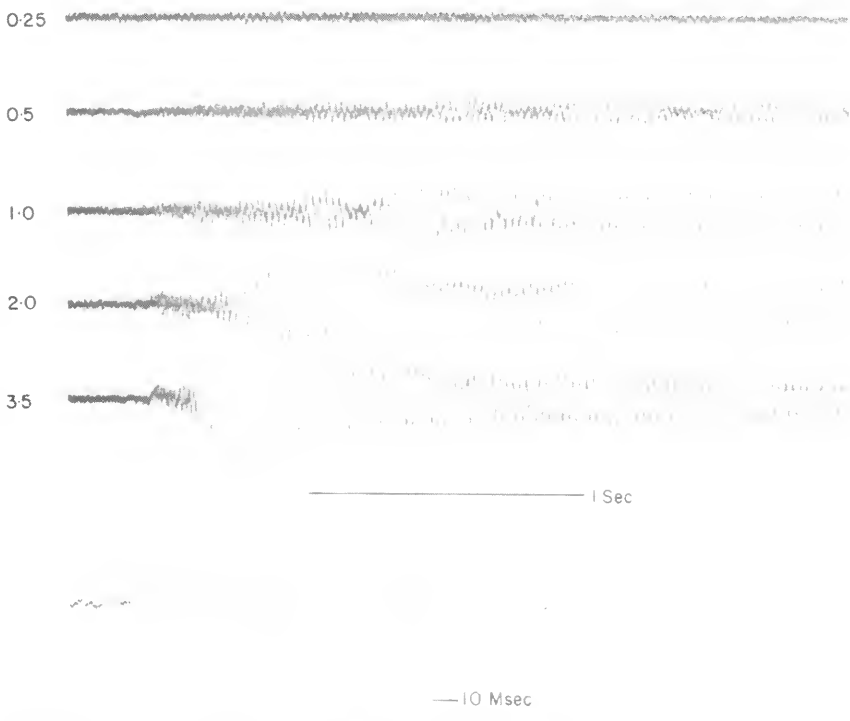


Figure 27. Olfactory waves in response to amyl acetate at various flow rates. Odor pulled through nares at constant rate with suction applied to the cannulated trachea. Values of flow rates in 1/min. Bottom record shows beginning of electrical nerve activity followed by wave activity in response to odor stimulation.

ceptors vary in their response to various odors. This information is greatly needed before any good theory of olfactory stimulation can be constructed.

Autonomic Effects. The nasal area is innervated by sympathetic and parasympathetic fibers of the autonomic nervous system (fig. 28). What influences do these fibers have upon the olfactory response of the receptors?

One may record from a small ethmoidal nerve bundle that carries sympathetic fibers. When the olfactory cells are activated by odors, the sympathetic activity decreases (fig. 29). If the odor concentration is increased so that the trigeminal as well as the olfactory nerve fibers are activated, then the sympathetic activity increases. The sympathetic activity can also be increased by pinching the foot, touching the eye, etc.

If one electrically stimulates the cervicle sympathetic nerve of the rabbit, the response of the olfactory area to odors greatly increases in magnitude (fig. 30). During fright, when the sympa-

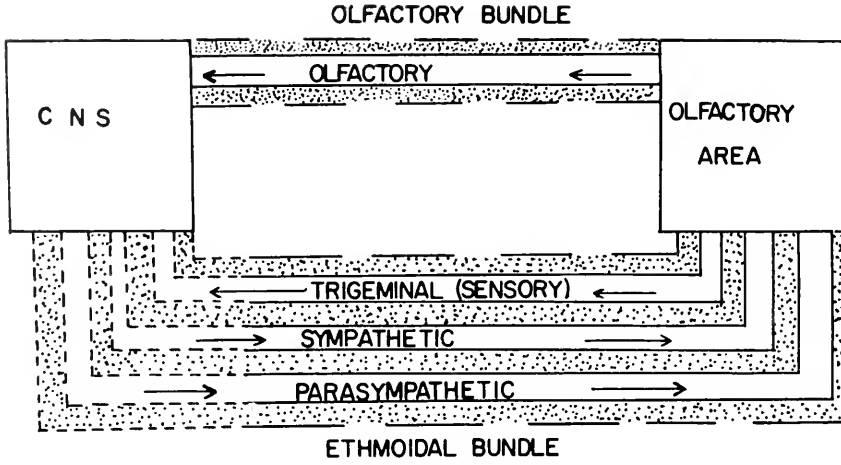


Figure 28. Schematic showing various nerve fibers important in the response of the olfactory area to odors.

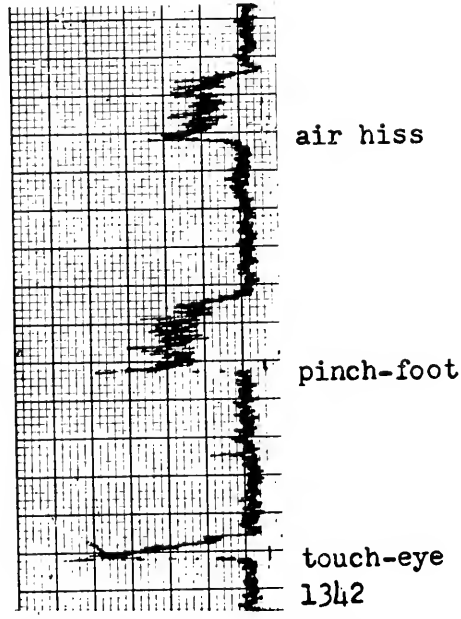


Figure 29. Integrated electrical activity of central end of ethmoidal nerve in response to various stimuli. Time scale: 1 unit = 20 seconds.

thetic system is active, an animal may be in a better position to detect odors because the olfactory threshold has considerably decreased. Since the effect of sympathetic activity on human olfactory thresholds has not been studied, it is not known how important such activity is in human odor detection.

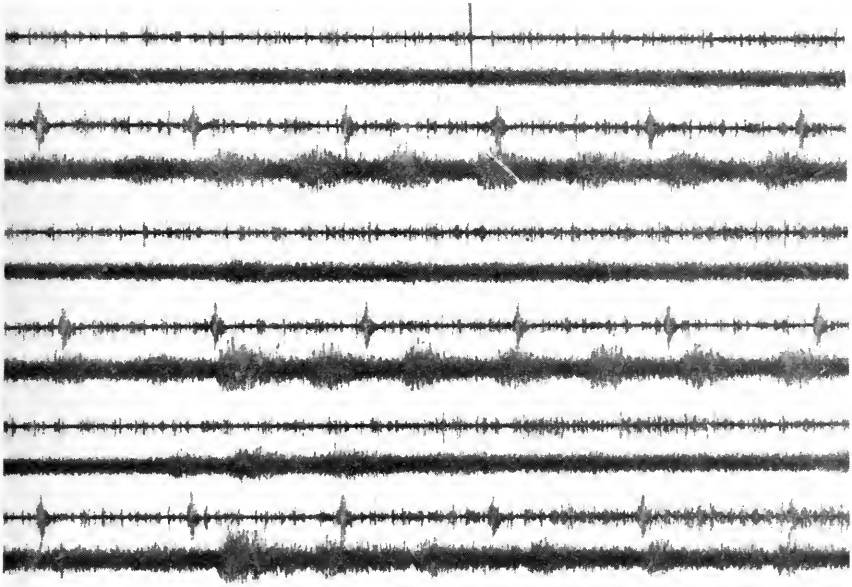


Figure 30. Olfactory nerve activity (bottom trace) in response to odors before and after ethmoidal nerve activity (top trace) is increased by 30/min electrical stimulation of cervicle sympathetic nerve. Records 1, 3, 5 show response to phenyl ethyl alcohol, orange, and amyl acetate odors before stimulation, and records 2, 4, 6 show response to same odors after stimulation.

Conclusion

The olfactory cells respond to a presented odor in less than 0.25 second. These cells respond to a large number of odors in very low concentrations. In fact, many odors are of less concentration than enzymes found in living cells. What special properties may one attribute to the olfactory cells to account for their unusual properties of molecule detection? Do the cells preferentially adsorb the odorous molecules in order to concentrate them on the surface of the hairs? Must the odor penetrate the aqueous layer covering the tissue? In answering these questions, one must take into account the fact that the bare nerve endings of the trigeminal system also respond within a second or two after odor presentation. No special histological structures are known to exist for these nerve endings. It has recently been shown that a layer of aqueous solution spread over the olfactory tissue does not interfere with the trigeminal ending's response to odors!

How does the olfactory system differentiate odor qualities? Most theories of olfaction assume specific receptors for each

group of odors. Unfortunately, the existence of different types of receptors has not been experimentally verified. The differentiation of odor qualities may also be explained by different spatial and temporal patterns of response initiated by a homogeneous population of receptors. For example, inhaled coffee aroma may pass over the billion olfactory hairs and the particular chemical in the aroma that binds strongest to the receptors will be adsorbed to those that it passes over first. The other chemical constituents will be adsorbed in order of their binding strength until the weakest is adsorbed last and at a location distant from the site of adsorption of the first chemical of the aroma. In this way a spatial and temporal pattern of response can be initiated that essentially analyzes the particular aroma in a manner similar to that of a vapor fractometer. However, little experimental evidence exists that allows the theorist to determine the actual mechanism of odor quality discrimination.

To determine the answers to questions concerning the wonders of the olfactory appartus, systematic experimentation must be performed on the olfactory tissue itself. The various parameters involved in odor stimulation need to be determined. Modern biophysical tools should be employed and a serious study should be carried forth over a period of many years. The nature of the stimulus and the delicacy of the olfactory tissue have made this sensory system the most expensive and most frustrating of all sensory systems to study.

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Discussion

DR. E. M. MRAK (University of California):

Dr. Beidler, I am not sure that I got this straight but would I infer that a pleasant stimulus during dinner would make me enjoy the aromas of good food better—what I have reference to is the stimulus of good music, etc.?

BEIDLER:

I would not like to carry it over to humans but in the case of animals I do not think that they pay too much attention to their olfactory senses except in a very crude meaning. I would not like to carry this analysis over too strongly from the wild animal to man. However, I do not think that music in the room or any other stimulus would have too much effect.

MRAK:

What about noise in connection with the taste panel? Would that cut down on the taste acuity?

BEIDLER:

I am not a psychologist, but I would be willing to agree that it would. The threshold that we determine is the lowest one. Of course, when one deals with the central nervous system, other factors emerge.

DR. A. J. HAAGEN-SMIT (California Institute of Technology):

I would like to ask if any experiments were performed using vitamin deficient animals. I remember a publication by some Frenchman which pointed out that if rats were deficient in vitamins, they could not smell the difference between two subjects. I think it would be a rather interesting study, and therefore I wonder if that has been done.

BEIDLER:

I don't know of any such study in connection with vitamins, but there have been some in connection with hormones. These hormones are related to the sex hormones (and the relationship of odor with the monthly cycle).

DR. F. J. PILGRIM (QM Food and Container Institute):

I would like to comment on Dr. Beidler's earlier statement. I think the very fact that flavor appears to be so important in the acceptance of foods contradicts his statements that odor is not very important in man.

DR. L. B. SJÖSTRÖM (Arthur D. Little, Inc.):

I don't know whether you covered this, Dr. Beidler, or not, but I would like to have you comment further on it for my own benefit. You did mention that you got responses with inspiration. We notice, in examining certain chemical compounds, smelling them, that the odors fade rather rapidly. You take an inspiration and sniff and then on the second and third sniff you get no response. Do you have any explanation for that?

BEIDLER:

I think the question concerns itself with the adaptation to certain types of molecules. Adrian has shown that if you go into the olfactory bulb, in the central nervous system, you do get complete adaptation. Therefore, even though we might record that there is no adaptation, the animal might not be able to smell. There is difficulty in using cross-adaptation experiments in the mechanics of taste and odor. They are not reliable.

QUESTION:

When you were observing the patterns you received as a result of a given stimulus, you used a simple detector to determine a response curve which would merely account for amplitude variation. Are frequency variations significant and, if they are, have you disregarded them?

BEIDLER:

I think I answered that question. Frequency is important as far as response is concerned, but the detector analyzes frequency, inasmuch as it is the total number of impulses. The time constant of the integrator is important. In some of the recordings I had a time constant of 3 seconds, and there you do not see any initial adaptation.

QUESTION:

I also have a question for Dr. Beidler. I was wondering if perchance he had run any blank controls?

BEIDLER:

Yes, we do have a series of experiments on that, and I shall be happy to talk to you later about them.

DR. J. HARTMAN (Cornell University):

I address this question to the group as a whole. Dr. Beidler has touched upon the point that salt may be conceived as loose ions in association with proteins and that this might be adequate for salts, and might possibly be adequate for acids, but that it would be stretching the point for sugars and most certainly for bitter substances. Also, in the case of the many things that we perceive as odors, we have to have something different from this idea. Therefore, I am wondering what the current status is with regard to an acceptable theory of olfactory perception. Do we have any adequate explanation of how we can tell the hundreds of different odors as distinct entities?

BEIDLER:

The theory I gave was with regard to electrolytes, but I really believe you have the same sort of thing with non-electrolytes. One molecule probably will combine both ion and non-electrolyte properties at the same time, because we have seen that one taste cell can respond to sugars, acids, salts, and bitters all at the same time. I think that speed of response points to the same type of mechanism. I do not think that the stimulus needs to get into the cell.

I think that in olfaction there are several theories. Mullins has tried to do some work using thresholds of homologous series and has come out with some idea of what is going on at the surface.

There are difficulties with all theories because we have no information with respect to how the receptor works. We cannot propose a theory unless we know whether the molecule has to go through a water layer or not.

DR. S. METTLER (Armour Research Foundation):

For the past 3 or 4 years we have been sponsoring fundamental research on odor perception and, in our course of study, we have found that 99 percent of the theories that we have run across are just theories; therefore, we wonder about the validity of them.

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Dr. Beidler, during the past 3 years, has brought research on odor perception almost up to the position of research on taste perception. There has been almost a 40- or 50-year gap in experimentation of this type, and this has been due to the fact that there has been much difficulty in connection with odor identification. Therefore, I want to commend Dr. Beidler for his progress in this field.

I think that these new theories tend to clutter up the light on this subject and what we need is more research work and less theory.

BEIDLER:

Of course, I have an interest in this question myself—the question of theory. In these days, when we do experiments, we find that our notions do not fit anymore. However, the concept that Dr. Mettler has proposed and which someone else has written about is something that sounds very reasonable to me. I would say that the spatial configuration of the molecule is something that is very important—the type of site and so on.

DR. L. FERGUSON (Howard University):

I just heard, in a conference we had in Washington recently, that a Dr. Johnson of Georgetown University is working on this. I understand that one of his students made this study.

PILGRIM:

Dr. Beidler, you pointed out that the sour response was not adequately predicted by the pH or molarity of various acids. Have you attempted a multiple regression on these to see whether the two together will predict the amount of response?

BEIDLER:

I think that in most cases where you get a regression relationship between pH and response you always have the variable factors. Our relations are very good if you compare them with what goes on in an ion exchange column.

HAAGEN-SMIT: I only arise to remark that the infrared idea is not dead. I believe that about 2 or 3 months ago a new paper came out on the effect of the infrared vibrations through a coupled vibration to the receptor molecule in the little hairs. It was further explained that the receptor molecule in the olfactory system was a pigment and that this would undergo vibrations stimulated by the molecule. In such a case, this would require

less energy than would an ordinary chemical reaction. This is not impossible; however, I do not wish to promote the idea, but it is something worth thinking about since you mentioned the matter of the small amount of energy necessary in these reactions.

Now, I would like to take issue with the other gentleman on the question of too many theories. I think that we have to distinguish between a theory and a fact. The trouble with most theories is that they are too generalized. They only hold for a small group of organic compounds and do not explain all of the odors that occur in nature. I think that we have to accept a different theory for sulphur compounds and another theory for other types.

MRAK:

Dr. Tressler, you have heard all of this; therefore, what is the Army going to do about it?

DR. D. K. TRESSLER (QM Food and Container Institute):

It seems to me that we need all of this experimental work, including the theories. This argument as to which is more important, the matter of having the theoretical explanations or the actual experimental work is like the argument of which came first, the chicken or the egg. I feel that the answer is that we need both.

Measurement of the Qualitative and Quantitative Attributes of Flavor

FRANCIS J. PILGRIM AND HOWARD G. SCHUTZ

Quartermaster Food and Container Institute

The words "taste" and "flavor" have various meanings. For our purposes, taste is restricted to the gustatory sense. Flavor is the complex of sensations produced by an item that is normally considered edible. It may and can include any or all of the specific senses of taste, odor, touch, warmth, cold, pain, and kinesthesis; it may even encompass vision and audition. When considering the chemistry of flavors, we may reasonably restrict the meaning of flavor to odor and taste with occasional components of warmth, coolness, and trigeminal or pain sensations.

A direct stimulus to a sensory end organ can produce two types of responses in a person, depending in part on his set or predis-

position to action. One response is affective; i.e., feeling or hedonic tone; the other is discriminative or judgmental in character. With regard to foods, the affective response is commonly measured in terms of preference, but discrimination is the appropriate response for flavor analysis. Preference is commonly used to predict the acceptability of foods, and directed discrimination should not be introduced because it may lead to biased results. On the other hand, in any judgmental task, the subject must be oriented to minimize affective responses and to maximize discrimination.

Although the response to a stimulus possessing flavor characteristics may be measured physiologically, such as the lacrymatory response to onion vapors, flavors are much more commonly dealt with in terms of verbal behavior. Therefore, the first requisite is to have a set of appropriate verbal responses to be applied to a substance that produces a sensation of flavor. *Appropriate* means, first of all, that the responses be, or symbolize, qualitatively distinct attributes of flavor. Secondly, there must be a procedure for quantifying the responses. If these requirements are met, we have a system by which we may hope to measure the quantitative and qualitative attributes of flavor. The methods for establishing such a system are the major concern of this paper.

Obviously, the starting point of any descriptive or classificatory system is common everyday language. The most frequently occurring attributes will be "tagged" in this way and they are likely to be at least *nearly* correct. However, two types of errors are likely to arise: one from ambiguities in the meanings of words; another from inability to specify the true number of categories that exist. The latter problem still exists with regard to odors; and, since odor is, in many people's judgment, the most important part of flavor, the problems and suggested solutions will be mainly in terms of odor.

The problem of classification of odors is evident from the fact that various investigators have proposed various systems (See *(1)* and *(2)*). Linneaus had 7 categories; Rimmel had 18; Zwaardemaker, 9; Henning, 6; Erb and also Crocker-Henderson, 4, and Foster, 24 plus 8 touch and other sense qualities. It has been recognized and often stated that none of these systems is fully satisfactory.

Some tried to place the categories into a system, such as Henning's prism, figure 1, or Foster's scheme, figure 2. Where did the words come from? Probably from common language, intuition, educated guesses, and possibly one other useful tool for pre-

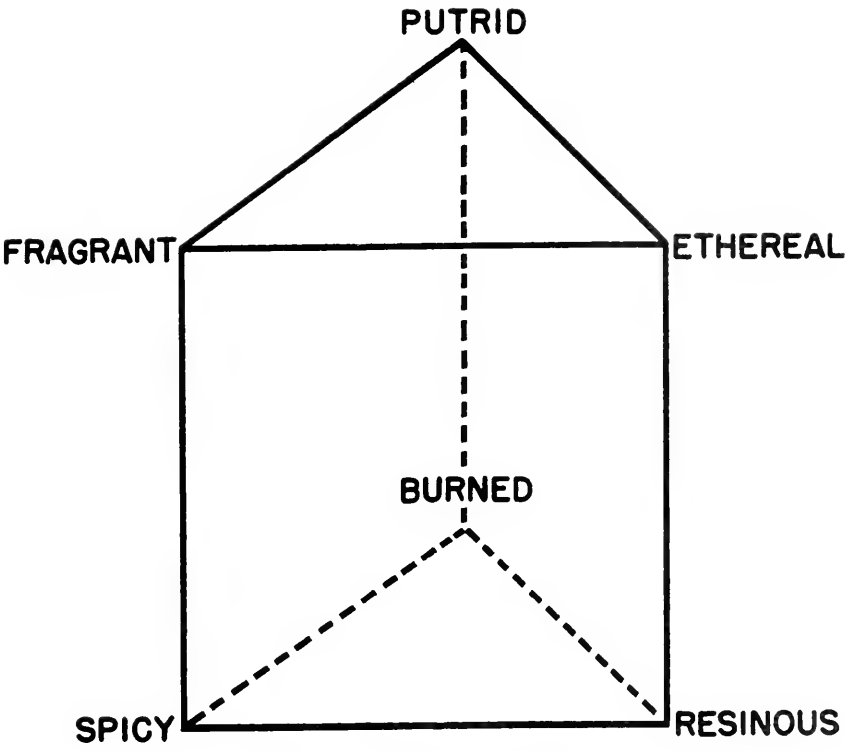

Figure 1. Henning's odor prism.

liminary work—the free response. The latter consists of having a number of people each supply as many descriptions as possible for each of a number of stimuli, which for odors might consist of foods, extractives, or pure compounds. The major problem is that one ends up with too many words, some being synonyms and some being inappropriate. A little bit of the problem can be overcome by giving actual examples and saying: "This is typical of what I mean by such and such a word." This may increase uniformity of judgments by a group of people, but it can also go wrong if the words really mean something else to them. Therefore, starting with past procedures and systems, we embarked on the present approach to odor classification and flavor analysis.

It was believed that factor analysis, in which many variables are dumped into the hopper, so to speak, and the basic variables or factors are extracted, could lead to a useful classification system. Although this analysis has not yet been performed, the results of certain other analyses lead us to believe that quantitative flavor analysis along with sound statistical procedures is feasible, even when using subjects or observers without specific training on flavors.

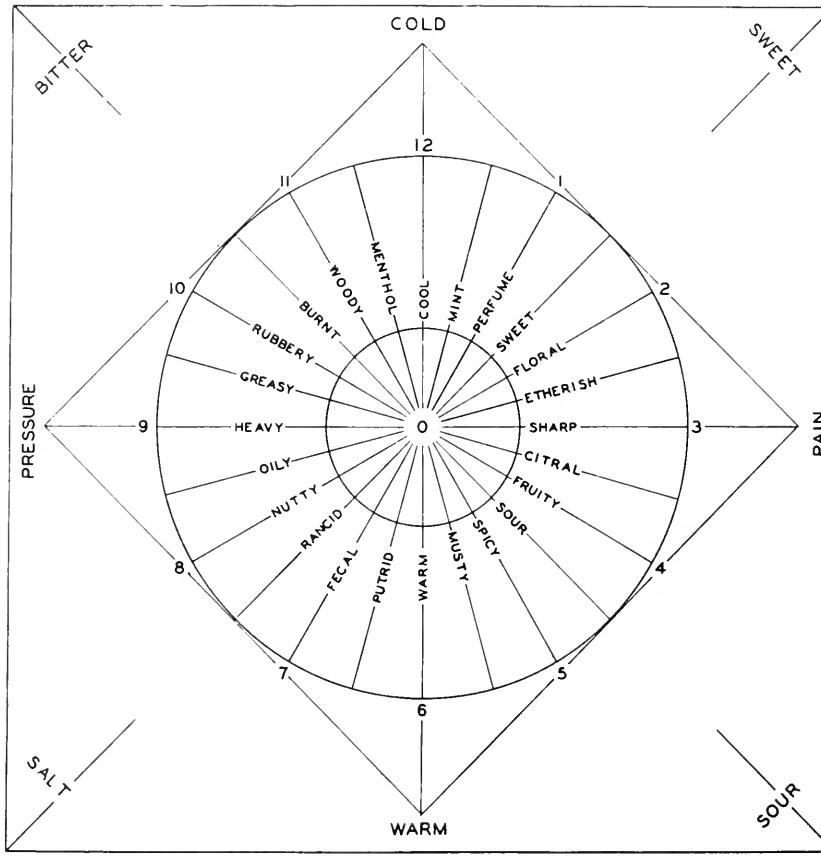

Figure 2. Foster's odor classification system.

From among the various classification systems we chose the words or attributes common to two or more of them, with the restriction that words not common to our language such as "alliaceous" or "empyreumatic" would not be used. In addition, words from any one of these systems that we presumed might be meaningful were included; and some words were obtained in a free-response situation employing 5 odorants and 5 people. The people responded independently of one another. We believed that people could respond to a number of descriptive words at one time without fatigue. The final list consisted of 27 words or attributes. One of the 5 random orders in which the words were used follows: rancid, metallic, acrid, fruity, tarry, heavy, putrid, etherish, sour, sweet, musty, warm, pungent, woody, fragrant, garlicky, flowery, burnt, dry, medicinal, fishy, cool, spicy, smoky, oily, goaty, sulfurous.

With so many attributes to be dealt with by the subject, we first considered restricting the response to a simple binomial type of quantification; i.e., *Yes* and *No*, the attribute is present or absent. However, several preliminary studies showed that people could rate all the attributes on an intensity scale of 9 categories, 5 of them anchored, similar to the following form:

none slight moderate strong extreme

The odorants were chosen on the basis of: (1) definite differences as to kind of odor; i.e., the attributes they were judged by the experimenters to possess; (2) similarities of some, such as a homologous series of alcohols and some moderately similar chemical structures; (3) availability in reasonably pure form. The following 30 odorants were used: ethyl acetate, amyl acetate, benzyl acetate, benzyl benzoate, methyl salicylate, butylamine, butyric acid, benzaldehyde, vanillin, guaiacol, eugenol, isosafrole, coumarin, *l*-menthol, geraniol, *p*-dichlorobenzene, pyridine, 2-picoline, skatole, methyl ethyl ketone, diethyl sulfide, methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 1-pentanol, 1-hexanol, 1-heptanol, and 1-octanol.

The 20 subjects, 10 men and 10 women, were professional employees of our Institute, including chemists, psychologists, home economists, food technologists, publications editors, and others.

For each session a subject sat in the small olfactorium shown in figure 3. An exhaust fan drew off the odor from around the subject and also prevented it from diffusing into the room. The subject opened the bottle containing the odorant and smelled it as often as he felt necessary to rate it. There was a 1-minute interval between odorants. In the first 4 sessions, the subjects rated 15 odorants per session for preference or overall intensity. Then for 27 specific attributes, each subject rated 5 odorants per session for 6 sessions. Each odorant and attribute list was randomized for each subject in order to average out time effects. Five odorants were selected for repetition in a final session, again with randomized orders of attributes and odors.

The statistics that support the contention that people can adequately and appropriately perform flavor analyses are, in the main, correlation coefficients. The correlations are between two sessions for the same odorant, between pairs of odorants, and also between sub-groups of people for the same odorant.

First, the sums of ratings over all subjects for each of the 27 attributes and for preference and overall intensity were obtained for each odorant in the 6 regular sessions, and for the 27 attributes in the final session for the 5 repeated odorants: menthol,

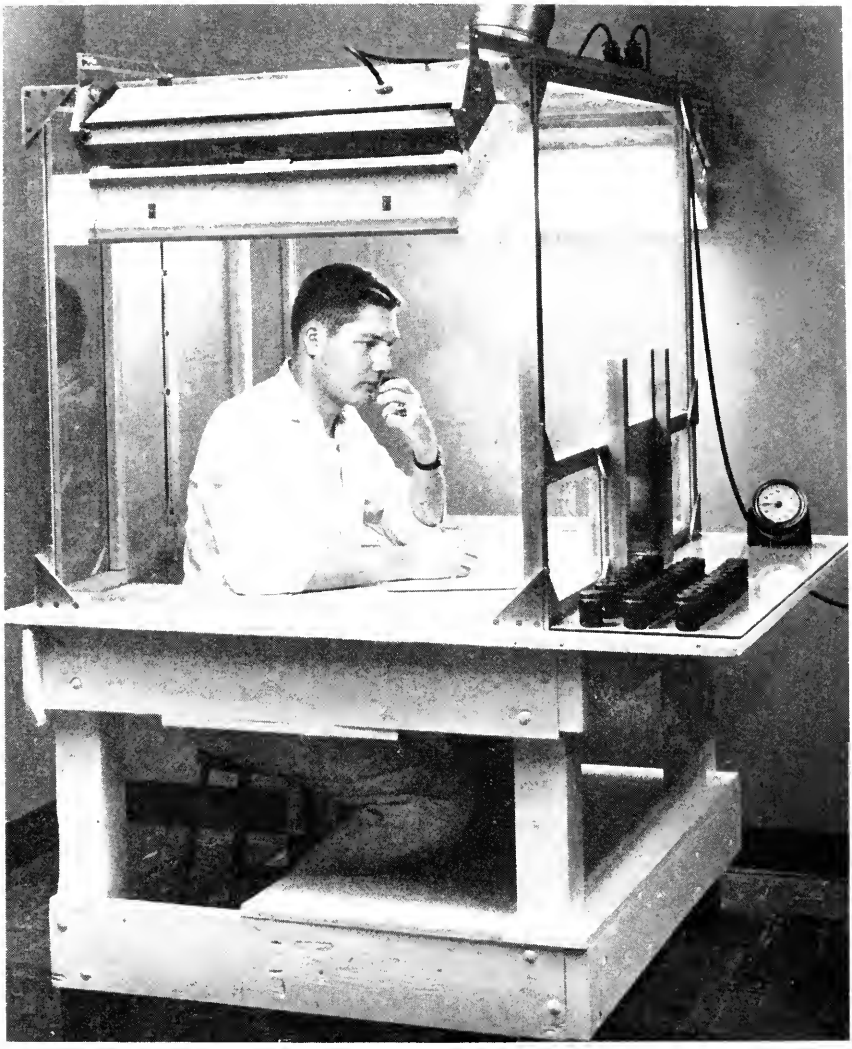

Figure 3. Olfactorium for presentation of odorants to individual subjects.

p-dichlorobenzene, eugenol, butylamine, and benzyl acetate. The sums of ratings were transformed to standardized scores so that each attribute had the same mean and variance. The correlation coefficient *(r)* for various pairs of odorants was computed, using the transformed sums of ratings of the attributes, including the original and repeat of an odorant as a pair. The latter is a reliability coefficient. The correlations ranged from .71 to .92, with an average (by Fisher's *z* method *(3)*) of .82. These reliability coefficients are quite high, demonstrating that people as a group can repeat their ratings of the intensities of a number of odor or-

flavor attributes of a substance. The fact that they can demonstrate this high reliability on 5 distinctly different substances permits us to generalize the results and predict that they would demonstrate a similar degree of reliability for other substances as well.

Could this be a spurious reliability just because people behave in the same way toward all the compounds; i.e., have a stereotyped behavior with little real discrimination? We selected pairs of odorants that should not be positively related: menthol *vs.* butylamine and vanillin *vs.* guaiacol; the correlations were —.47 and .01. These show that people as a group rated these compounds in distinctly different ways.

The lack of correlation among several pairs of compounds has an important implication. It suggests that there are some basically different and distinct dimensions to odor that can be isolated by the techniques employed in this study. Thus, it appears that the original purpose of the experiment can be realized: an odor classification system can be established with these techniques and factor analysis.

Remembering that each of the 27 attributes is assigned one of 9 degrees of intensity by each subject, and combining the conclusions of the first 2 sets of data, we can infer that people discriminate differences of a qualitative nature in a reliable quantitative fashion.

There are, however, some differences among people as well. The correlations between males and females for a given odorant are generally lower than the corresponding reliability coefficients. Inspection of their ratings showed that the women tended to use the touch or feeling attributes, such as cool, warm, dry, and heavy, more often than men. This is in accord with general findings in psychology that women respond to the environment with more emotional behavior than men.

The conclusions drawn thus far are necessary to establish a method of flavor analysis. However, they are hardly sufficient. Flavor chemistry can progress effectively only if there is a relationship between the sensory percept and some physical or chemical characteristic of a given substance. Certain relationships between odor or taste and chemical or physical variables have been discussed by other investigators *(2)*. Among these are chemical structure, surface tension, lipid solubility, and infrared adsorption. Further analysis of the data after the factor analysis may help determine which, if any, of these are important in odor.

In the meantime, let us examine an homologous series—the primary, normal alcohols from 1 to 8 carbons, plus isopropanol. The

Table 1. Correlations of attributes for homologous alcohols (Normal, primary, except for isopropanol)

No. of carbons	1	2	3	3-iso	4	5	6	7	8
1	....	.32	.22	.07	—.35	—.61	—.40	—.34	—.31
2	.32	....	.78	.62	.03	—.01	—.36	—.39	—.38
3	.22	.78	....	.79	.18	—.03	—.22	—.48	—.34
3-iso	.07	.62	.79	....	.34	.29	—.19	—.20	—.11
4	—.35	.03	.18	.34	....	.55	.44	—.07	.12
5	—.61	—.01	—.03	.29	.55	....	.40	.47	.34
6	—.40	—.36	—.22	—.19	.44	.40	....	.34	.52
7	—.34	—.39	—.48	—.20	—.07	.47	.34	....	.70
8	—.31	—.38	—.34	—.11	.12	.34	.52	.70	....

correlations among these alcohols are shown in table 1. In general, the correlations are highest for adjacent members of the series; they decrease as the difference in number of carbons increases until the alcohols that are farthest apart are actually negatively correlated.

Among the highest correlations, for other compounds, is the one between coumarin and vanillin, a .81, and the one between pyridine and picoline, a .80. However, there are compounds of similar structure that are not highly correlated and there are dissimilar structures that are highly correlated. Part of the latter characteristic is due to the great importance of affectivity or pleasantness-unpleasantness. Many of the words or specific attributes have this connotation in addition to their specificity. Other studies as well as this one have shown that affectivity is an important component of odors. It seems likely that a classification system will have to include this component as well as the specific attributes.

We have made a practical application to a specific problem of the quantitative analysis of flavor with factor analysis; it will serve to review the steps involved. The basic problem was the storage quality of various species and processings of frozen fish. We first oriented about 20 people concerning the general problem and their specific task. Then, they were served 3 or 4 samples of fish at each of several sessions. They were to supply as many descriptive words or phrases as possible that applied to each sample, and they were to avoid affective terms such as like, dislike, repulsive, etc. This procedure gave us about 50 terms, but many were used only once or twice, and others, such as names of chemicals, were specific to a given discipline. We eliminated these terms on the basis of our own judgment. Twenty-five terms were then listed on a rating sheet and the subjects asked to rate each of a number of fish samples for intensity of each attribute. The

data were then factor-analyzed to find out which words were merely synonyms or stood for 2 physical or chemical properties that vary together in fish. This permitted reduction to 15 attributes with just a few possible synonyms remaining.

These 15 attributes were then used in the main study in which the various types and kinds of fish were withdrawn from storage at scheduled intervals for flavor and chemical analyses. The flavor attribute ratings of intensity and the chemical measures were all inter-correlated for a factor analysis. This has provided information on what chemical measures and what flavors are important in the storage of fish and how the flavor changes are related to the chemical changes that occur. Inspection of the correlation matrix indicated that the analysis would yield meaningful factors. The first factor showed a high degree of relationship among various chemical measures of nitrogen, including amine, NPN, and saline extractable nitrogen. There are also factors relating attributes to each other and to chemical measures, and factors relating preference to other measures.

The results obtained so far from the odor analysis and the fish storage study support the idea, mentioned earlier, that nonexpert subjects, as a group, can reliably discriminate flavor qualities in a quantitative manner. Further, these qualities can be related to physical and chemical variables to aid research on the chemistry of flavor and to contribute to practical food technology problems.

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Discussion

QUESTION:

Have you encountered any difficulty in establishing and maintaining purity in your test stimulus substances, and have you observed large effects due to small traces of impurities that would seem to be out of all proportion to the concentration?

PILGRIM:

The answer is "no," because we did not try to. This was, of course, a preliminary study, and we were looking as much for

the ways in which people can describe odors. In this type of study, it actually does not matter whether you know what the odors are. I think that as long as you have a variety of them you should still be able to extract, by factor analysis, the basic categories of odorants. Of course, if your substances are impure, you will not be able to relate the categories or ways in which people perceive the odorant to the chemical structure. However, you will still have a classification system.

I would say that it will require several studies to determine first of all whether we can get a useful classification system before we will be too concerned with extreme purity in the compounds. We did attempt to get them as pure as possible, but then we did not take unusual precautions to refine them.

MRAK:

I was wondering if the question referred to carriers for these substances—were they carried or dissolved in alcohol?

PILGRIM:

The substances were in essentially pure form except, of course, where the odor was entirely too strong. In the latter case they were diluted in either water or odorless mineral oil.

BEIDLER:

How does your olfactory threshold for menthol compare to the temperature threshold in the same source? How do you know that you are dealing with olfactory perceptors when you use menthol?

PILGRIM:

We did not do any threshold studies. These were all subjective intensities. If a person indicated no intensity for a given attribute, obviously that attribute, at least, was below threshold. As to the attributes for menthol, anything positive, like "flowery" and "cool" would come out.

DR. J. H. NAIR (Thos. J. Lipton, Inc.):

This work, I take it, is the first of a series. You are perhaps starting with odor because that is so easily perceived. I mean that the sensitivity to olfactory stimuli is known to be very marked even in very dilute concentrations in the atmosphere. I take it that you have worked only with pure substances in the measurement of the intensity of the effect as perceived by the subjects, or have you used blends of these various substances?

PILGRIM:

Not intentionally. They were as pure as we could get them without using specialized techniques. This is the first of a series of studies that must be done on odor before we can actually hope to get a reasonably good classification system.

QUESTION:

I gather that you feel that much can be accomplished in quantifying the results so far as odor perception is concerned?

PILGRIM:

That is correct.

QUESTION:

Do you anticipate as good results with other attributes which are much more difficult to evaluate than the odor attributes of flavor?

PILGRIM:

Others are perhaps just as easily quantifiable. I believe that other people have done studies on touch perception in the mouth and have shown quite a fine degree of discrimination. One study that I know of had to do with feelings in the mouth in connection with the manufacture of dental plates, and there, again, a small difference can be discriminated. I believe that texture also can be made quantifiable.

DR. J. M. MCINTIRE (The Carnation Company):

When you get blends of compounds, do you anticipate being able to separate those by sensation or are you going to qualify them by some other manner?

PILGRIM:

An optimistic answer there would be "yes." Apparently, if people can (as has been shown from this preliminary data), describe different odorants differently, then obviously if two of them are present together they should be able to at least describe some of the attributes of each of the materials present. However, I will admit that this is probably a more difficult problem, and I don't think that we will have it solved *this* year.

QUESTION:

I would like to ask, since foods generally have mixed odors and flavors, what effect does the frequency and order have in the pre-

sentation of these various foods from the quantitative point of view?

PILGRIM:

I think that effects on discrimination itself are present, but they are not serious. If you allow a reasonable interval of one or possibly two minutes, depending upon the intensity of the substance, then I am sure you will have no trouble. Of course, we do know that there are always present in any measurements certain order effects; and, we know from the work at the Institute that, in terms of affective responses, such as preference, there are very marked effects. That is why best results are obtained when you have controlled orders.

Of course, you also know that we are proponents of the essentially mass attack on these problems—from 10 to 100 people being used in connection with any study.

Gas Chromatography and Mass Spectrometry

In the Study of Flavor

WILLIAM H. STAHL

Pioneering Research Division
Quartermaster Research and Development Center

Some of you are familiar with mass spectrometry and recognize it as a powerful analytical tool. More of you are probably familiar with gas-liquid partition chromatography and realize that it, too, is becoming an extremely useful and powerful analytical technique. However, when the latter is used as an isolative system together with mass spectrometry, the result is truly synergistic. It is my intention to outline how we in Quartermaster are using this combination in the analysis of odor, particularly as regards food odor.

Let us examine quickly the general method of investigation of an unknown odor as we practice in our laboratory, as indicated in figure 1. We have divided this up into 7 separate steps. Steps 1 and 2 are obvious to a group such as this and need no further discussion. Step 3 consists of prefractionation by bulb-to-bulb distillation of the composite odor at low temperature and very low pressure, allowing a crude separation of widely boiling components, so that when put onto the chromatographic column finer separations can immediately be achieved. Steps 4, 5, and 6 shall be discussed in more detail later. Step 7 is the ultimate goal, and we have not attempted as yet to progress in this scheme this far on any of our research.

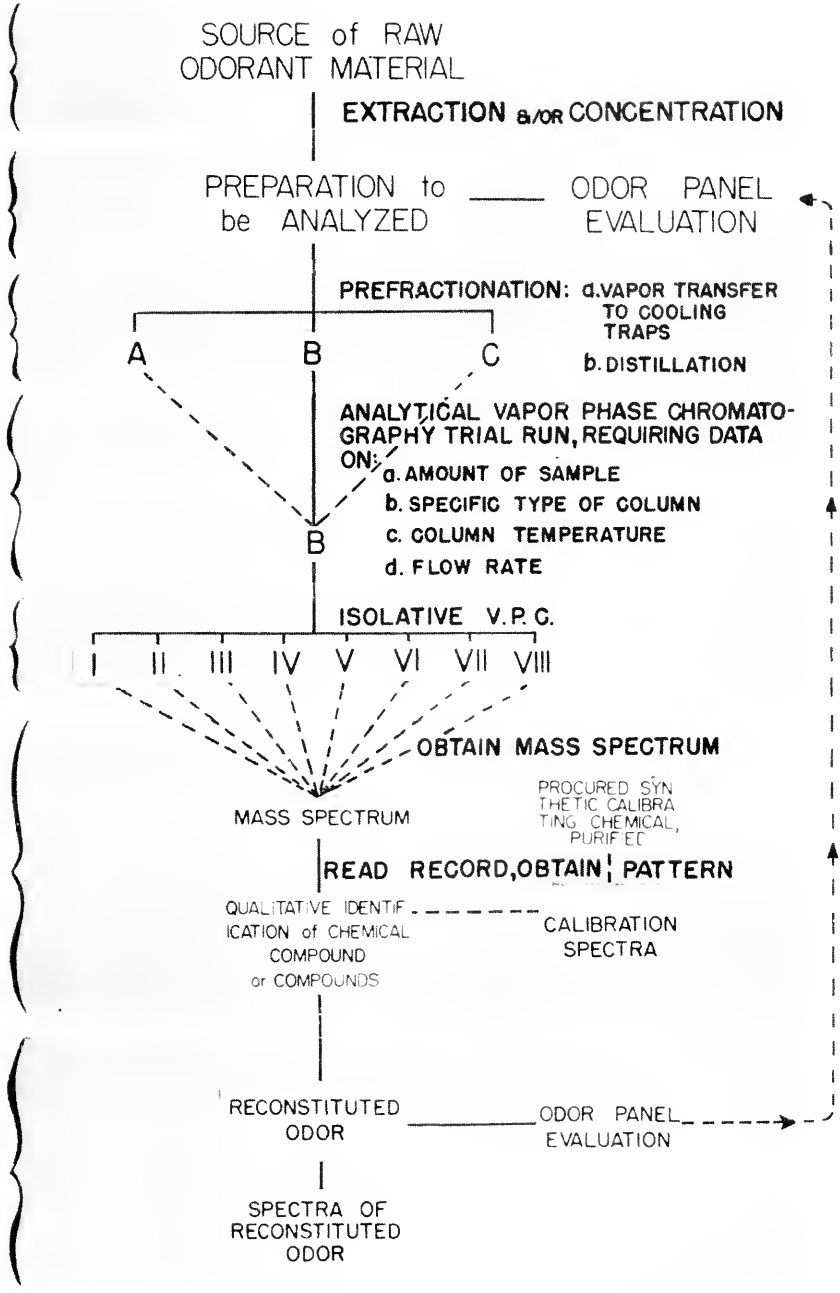

Figure 1. Scheme for investigation of an unknown odor.

To appreciate the experimental data on certain food odors, which I shall present subsequently, I would like to explain the principles involved and define some terms used in mass spectrometry. Figure 2 is a diagram showing the principal parts of a mass spectrometer. A sample of the unknown substance is stored in a small container at relatively low pressures, usually in the vapor phase. One might ask at this point—how small is a small sample? There are several ways one may put this: 1×10^{-5} mole is usually sufficient to record a satisfactory mass spectrum; another way is to say that it is that amount of volatile material that will exert 50μ pressure in a 4-liter bottle; one can detect as little as 1 percent impurity in this size sample. If certain adjustments are made to the spectrometer, this detection can be increased by 9 times. One further comparison—I shall speak about meat odor later, and therein one of the components of odor was calculated on a weight basis to be present as one part in 100 million parts.

To get back to the diagram, the molecules which are electrically neutral are bombarded by electrons, and some become positively charged ions and others are fragmented and become positively charged. They are accelerated to a high velocity and are sorted according to weight by magnetic means. As each beam of separated ions sweeps across the collector, its intensity is recorded. Figure 3 will show us the fragmentation pattern of a relatively simple molecule, acetaldehyde. It is noted that this molecule is broken up into 3 major positively charged fragments — CH_3^+, CHO^+, and a parent mass peak of $CH_3 \cdot CHO^+$. The abscissa is fragment mass while the ordinate reflects the comparative quantity of each fragment; thus this relationship is specific for acetaldehyde and no other molecule. Each chemical compound has a pattern unique to it.

Another device that aids the mass spectroscopist in identification of a compound is the use of the isotope ratios at certain key peaks. For example, in this compound there is a certain amount of 45 peak in relation to 44 peak, the 45 being due to isotope alone (1 percent). This device is also extremely useful in the identification of sulfur and halogen-containing compounds.

Another device that I shall subsequently mention is the use of a rearrangement peak. If we take a heptanal such as indicated in the equation below and bombard it with electrons, a very unusual thing happens. A molecule of water splits out and we are left with a C_7H_{12} fragment, which is not the normal type of break-

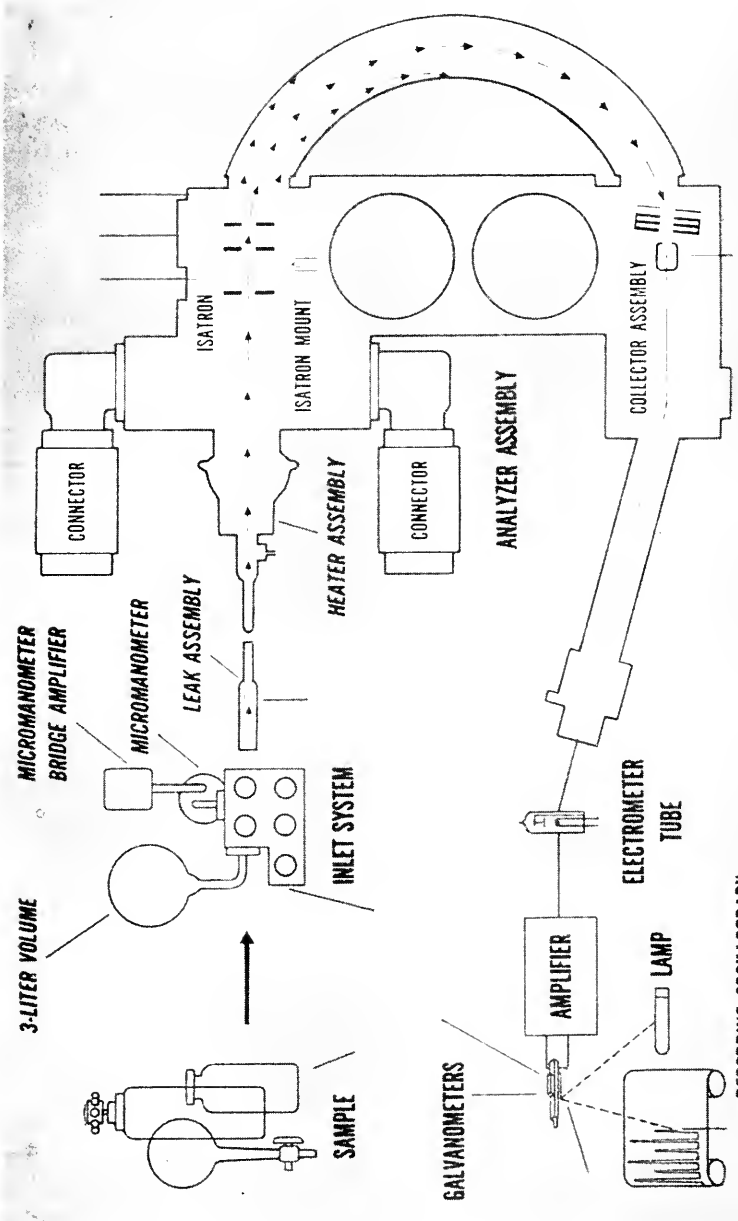

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Figure 2. Diagram showing principal parts of a mass spectrometer.

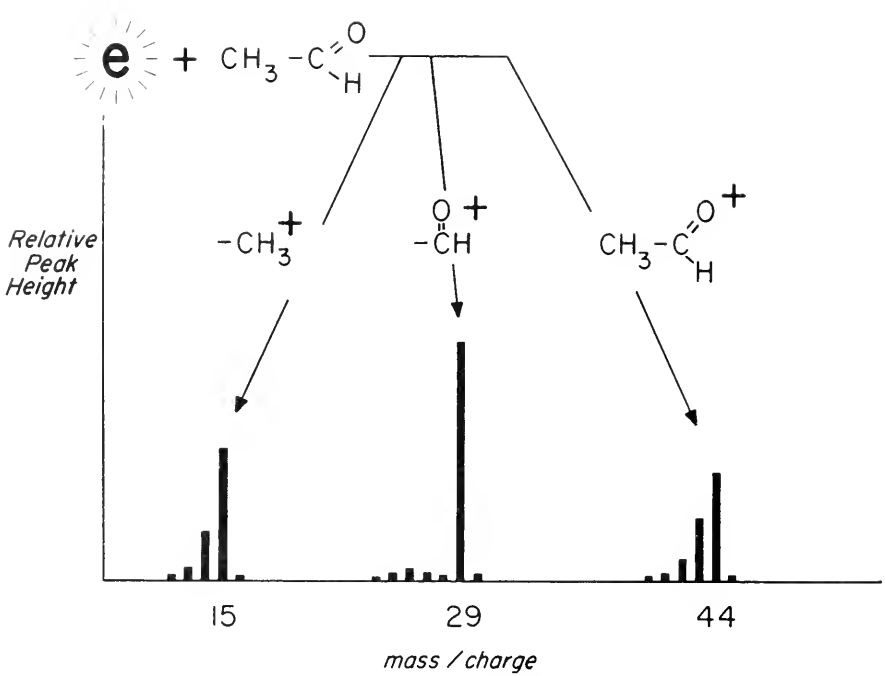
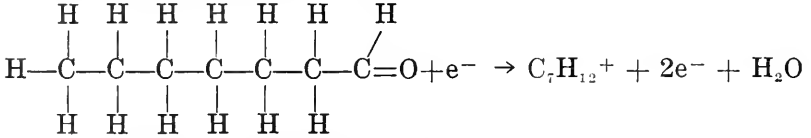


Figure 3. Fragmentation pattern of acetaldehyde under influence of electron bombardment.

down one might expect. We do expect, for example, to lose a CH_3, or a CHO, or a $CH_2 \cdot CHO$,



but in addition we get this rather unusual fragment which we term a rearrangement peak. Table 1 shows us that we might ex-

Table 1. Use of rearrangment peak

	n-Octane		n-Heptanone		n-Heptanal	
	m/e	%	m/e	%	m/e	%
M	114	6.7	114	13.0	114	2.0
M–18	96	0.0	96	0.0	96	17.0

pect a compound having a parent mass M of 114 to possibly be either the hydrocarbon n-octane, the ketone n-heptanone, or our aldehyde, n-heptanal. However, the fact that only the heptanal has any contribution to the parent mass — 18, which is that of

C_7H_{12}, allows us to diagnose with little doubt that our compound is indeed n-heptanal. Note that in this case the amount of C_7H_{12} fragment is present in approximately 8 times the quantity of the parent ion.

Now what are the limitations of an analytical type mass spectrometer insofar as our problem of quantitatively identifying food odors is involved? Under normal operating conditions, that is, without a special heated inlet system, data can be obtained on compounds up to molecular weight of 300; another way to put it is that one can run any compound whose boiling point at atmospheric pressure does not exceed 200° C. A C_{12} hydrocarbon is typical of the upper limits of usefulness of our spectrometer. A second and more serious limitation is the following: if a known mixture has perhaps 12 components, and one calibrates using peaks unique to each component, it is a relatively simple matter to obtain quantitative data. However, if it is a completely unknown mixture of 12 components, contributions will be obtained at all mass peaks, and you will be unable to make a start without simply assuming the presence of a certain compound and thereafter, by dint of laborious calculations, try and fit them into the pattern. Thus it is obvious that in the case of unknown compounds the certain identification is improved by the lesser number of compounds presented for mass spectrometric analysis. Again, if only a single pure compound is presented, and although you do not know what it is, a structural analysis of the spectrogram will allow you to predict what it is; this is followed up by comparison to a known standard spectrum, and the identification can be verified.

Thus it is obvious that for unequivocal proof of structure by mass spectrometry one needs an additional tool—one which will separate and isolate single or simple mixtures from highly complex mixtures. Such a tool has been found in isolative gas chromatography.

We have been fortunate in having Dr. Corse give a thorough presentation on the principle involved in the use of gas chromatography as an analytical technique. However, I would point out that we use this not as an analytical technique but simply as an elegant means of separation, and have added an isolative technique to it. If there is attached to the exit port of the chromatographic column a trap as is shown in figure 4 it is possible to condense a fraction as it comes off. If one either strings out more traps as we do, or uses one of several other designs for arrangement of traps, one can take off fractions into separate traps by suitable manipulation of valves or stopcocks. Since we are pri-

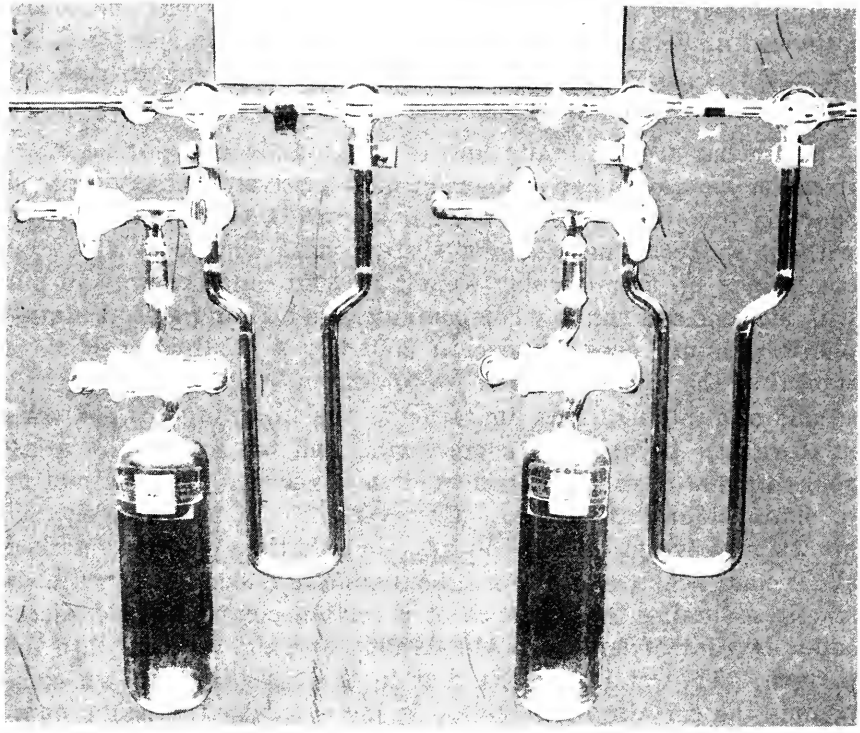


Figure 4. Gas chromatographic sample collection trap.

marily interested in presenting the contents of the trap for mass spectral analysis, we have a high vacuum system attached which allows us to pump out the carrier gas and leave only the condensables. These then are transferred by a bulb to bulb distillation into a gas sample bottle and subsequently run on the mass spectrometer.

Figure 5 depicts the type of apparatus we have in our laboratory for this purpose. There are two complete systems, designed specifically to fit our needs. We are developing a completely automatic high temperature, grease-free collection system as shown in the upper portion of the rack, but since we are interested primarily at this time in presenting a general method, we shall leave the specific design details for another occasion.

I would like to expand somewhat on the contrast in the use of gas chromatography alone and the use of the combined techniques of GLPC and MS. Since many of you are familiar with the technique of paper-partition chromatography, you recall that the technique utilized to prove the identity of a spot is that of substitution of a suspected compound and measuring its so-called R_F value.

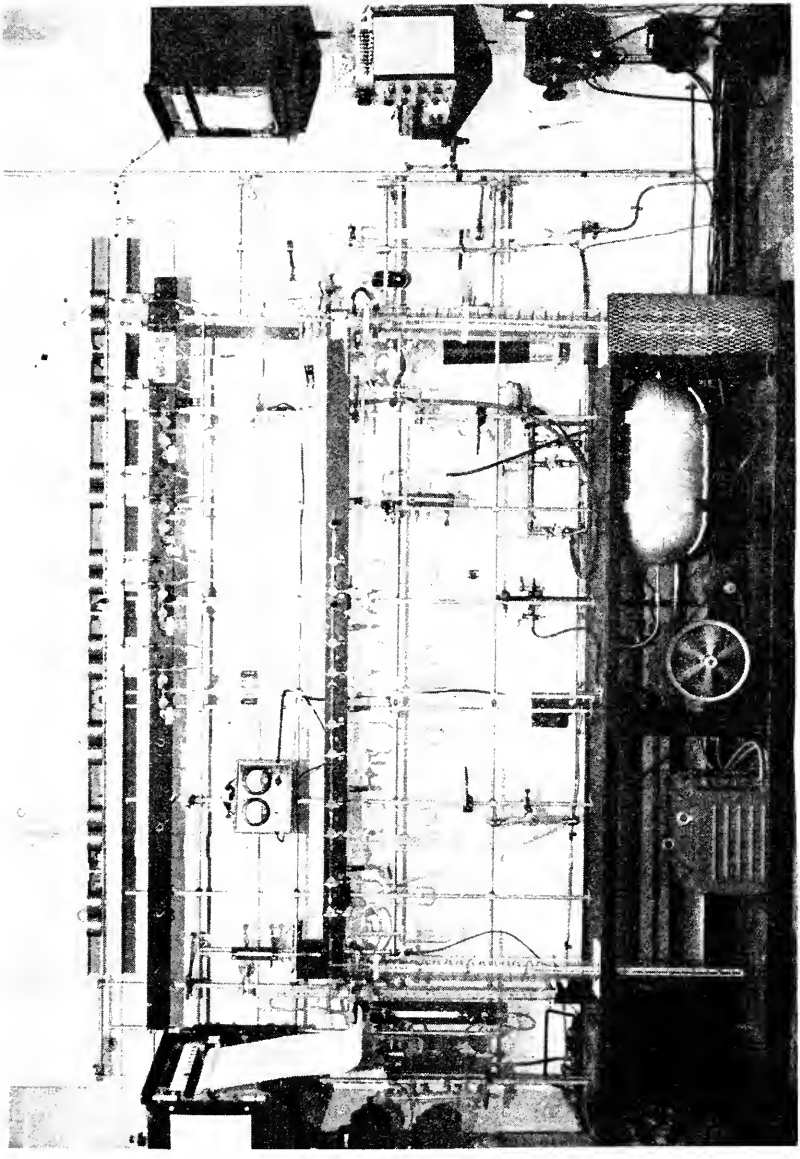


Figure 5. Gas chromatography apparatus.

This value is the ratio of the distance it migrates in a particular solvent system to the distance of the solvent front. If the suspected compound does match the R_F of the unknown, the same two compounds are then tried on a different solvent system and if again they migrate similarly, the chances are greatly enhanced that you have found the correct compound. As in paper chromatography, gas chromatography must rely on a similar procedure for the identification of an unknown. This may, for some specific problems, turn out to be a very difficult job.

A further complication in the sole use of gas chromatography comes from a consideration of the following. It is now well known that members of a homologous series usually separate out neatly one from another as is indicated in figure 6, which shows

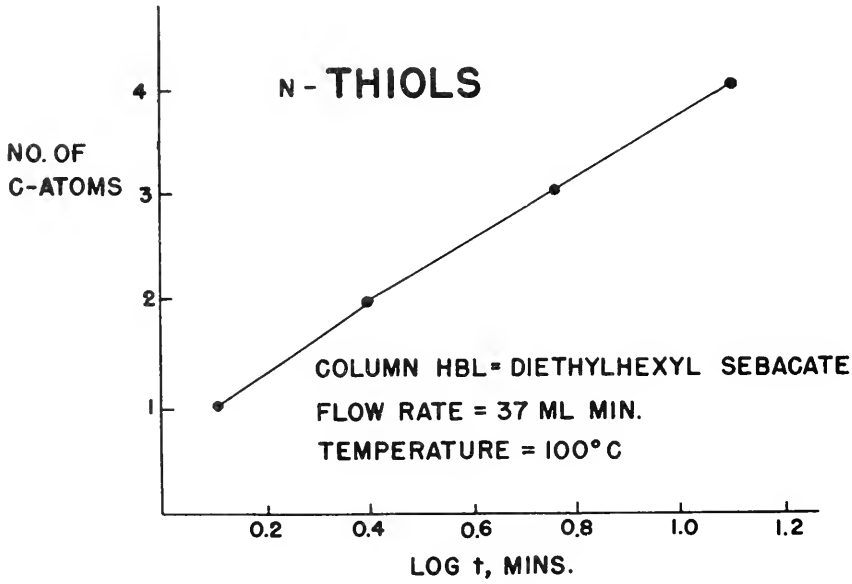


Figure 6. Log of retention time vs. no. of carbon atoms.

data of a homologous series of thiols or mercaptans obtained in our laboratory. In this particular case, the plot is that of the log of the retention time (T_r) vs. the number of carbon atoms in the molecule. If we were to replot this in terms of the detector and time, a neat series of peaks would be obtained, each separated from the other after returning to the baseline. However, now imagine the case of a complex odor, where one will get a composite of heterologous molecules. This may be illustrated in figure 7 which is the chromatogram of the —110° C. condensate of

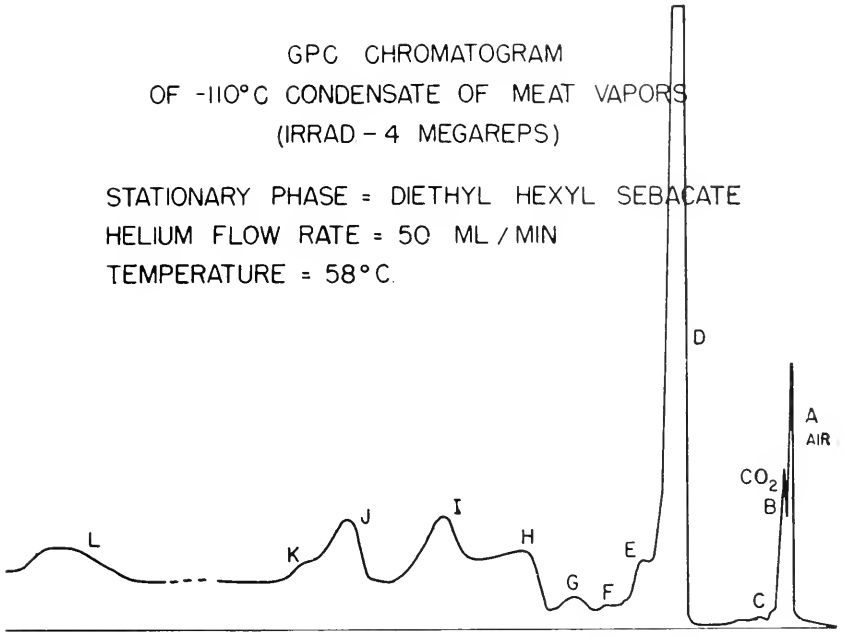


Figure 7. GPC chromatogram of —110° C. condensate of meat vapors (irrad. —4 megareps).

meat vapors. I shall explain the interpretation of this later. Suffice it to say that this cut contains sulfides, disulfides, aldehydes, alkyl benzenes, alcohols, and N-containing compounds. Thus you immediately see that there is a choice of several homologous series to use as calibrating materials. Also, it is evident that a further fractionation should be carried out until all peaks have resolved themselves to the baseline. This to me is a formidable task, for, as you shall see later, this represents only some of the 40 different compounds identified in meat odor.

Now if a mass spectrometer is available, and I realize that this is an expensive instrument that few in the food industry possess at the present time, then these two techniques can be advantageously used together, as I have pointed out previously. However, I would repeat that in this system of analysis, gas chromatographic equipment is used, not to identify compounds, but to act as a super-distillation apparatus, effecting separations that could hardly be achieved by any other means. Looking again at the diagram before us, we can either isolate entire peaks or groups of peaks, or "center-cut" individual peaks. This is indicated in figure 8. Peak A can be isolated *in toto*, and will represent pure A; but trapping total B would give a sample containing much C.

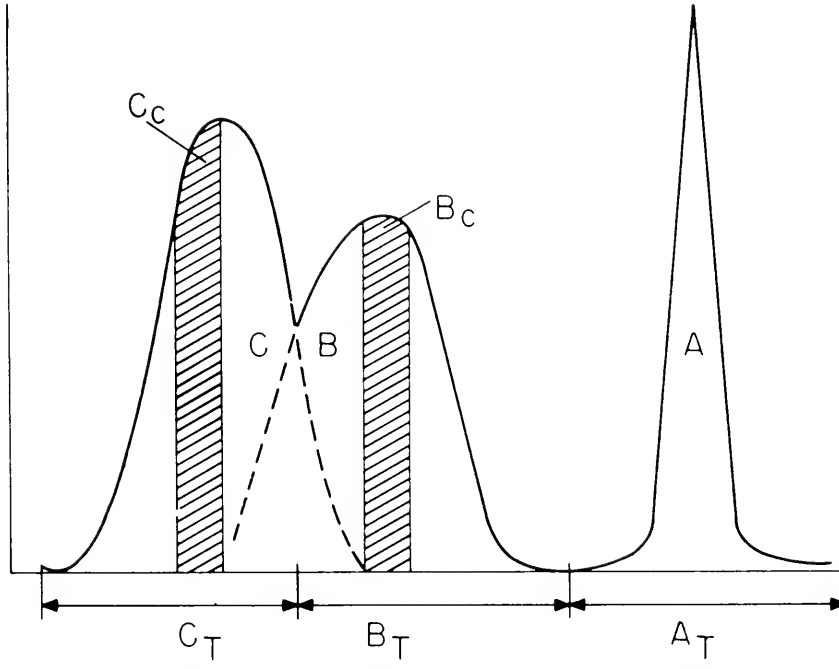


Figure 8. Graphical representation of total trapping vs. center-cutting.

Therefore, it is indicated that "center-cutting" would be worthwhile, and indeed, as the crosshatched area indicates, B center cut would be almost pure B and the same for C. Thus one is spared the additional task of looking for a different stationary phase that will better resolve these two.

Now I should like to present to you some of the specific results that we have obtained. While not directly concerned with food odor or flavor, it may be of interest to mention that we have applied these techniques to the isolation and identification of volatile compounds from glands of insects. This is a part of our Entomology group's program on the study of naturally occurring insect attractants and repellants. Two of the insects are well known to the food industry. They are the cockroach (and the species we studied ejected pure 2-hexenal) and flour-beetle of the Triboleum species which gives off a mixture of three specific quinones: methylquinone, ethylquinone and toluquinone. Other secretions of insects are under study in our Laboratories.

Some preliminary work has been done on odor components of whole roasted coffee bean, and the indications are that we can make progress on identification of these compounds. Whether we shall or shall not go deeply into a program of this kind has not been decided.

Our first effort after acquiring a mass spectrometer was a study on the gaseous emanation of the onion. Without going into detail I would like to present the essential results of this study (table 2).

Table 2. Compounds found in study on gaseous emanation of the onion (using mass spectrometry)

Compound	Relative amount
Propionaldehyde, Methyl alcohol	Very abundant
Propyl mercaptan	Abundant
Hydrogen sulfide, Acetaldehyde	Small
Sulfur dioxide, Dipropyl disulfide, Propyl alcohol, 4-Hexen-1-al[1], β-Hydroxy propanthiol[1]	Trace

[1] Suggested structure.

It is interesting to note that the dipropyl disulfide has a definite "sweet" odor typical of disulfides, and when simply combined with n-propylmercaptan, a creditable onion odor is produced. I would also point out that there can be no ambiguity as existed in the reports of earlier investigators. We make no claim that this work reports all of the volatile odors emanating from onion. However, no evidence was found of the existence of allyl propyl disulfide as reported by other investigators, despite the great care taken in processing samples and searching for this compound.

Finally, I should like to present our most recent work which has allowed us to improve the technique to the point of being able to make a qualitative and quantitative analysis of the vapor phase. As you are aware, no doubt, Quartermaster is interested in the radiation preservation of food. Often when certain products are given a so-called "sterilizing dose" certain odor changes are produced. Beef is in this category, and the odor change is on the unpleasant side. Since beef is an item of importance in the Army subsistence program, a study of the changes brought about by radiation sterilization has been undertaken, and we have been assigned a small part in the overall program. Our work, reported here, was restricted to the analysis of vapors over raw ground round steak.

Two of the samples for investigation were given minimum doses of beta radiation of 2 and 4 megareps. The radiation was administered by means of a 2 MeV Van de Graaff generator. A typical sample size was 200 grams.

To preserve the fresh meat and to avoid the side effects of spoilage, all of the meat samples were irradiated and analyzed within 36 hours after procurement and grinding. During this interim the meat was kept frozen.

The meat was irradiated at 20° C. so that free radical action initiated by the radiation would not be inhibited. Any thermal effects due to the radiation were minor, since the temperature of the patties rose only a few degrees during radiation. The samples were refrozen immediately after radiation until the time of analysis.

During the radiation, the meat patties were sealed in gas-tight bags of DuPont "Mylar" film which is essentially impervious to gaseous diffusion. Prior to sealing, the air was pumped out of the bags and an atmosphere of argon was introduced. The reason for this was to minimize any side-effects due to oxygen.

The general outline of our analytical technique consists first in concentrating the total vapors from the meat by condensation. This condensate subsequently is separated roughly by low-temperature vacuum distillation and then into finer cuts by means of gas chromatography. Identifications are based upon the mass spectra obtained from both the separations and the cuts.

Our system of concentrating and rough separation of meat vapor is simply a series of traps, each separated by high vacuum stopcocks. At the far right in figure 9, there is a 1-liter round-

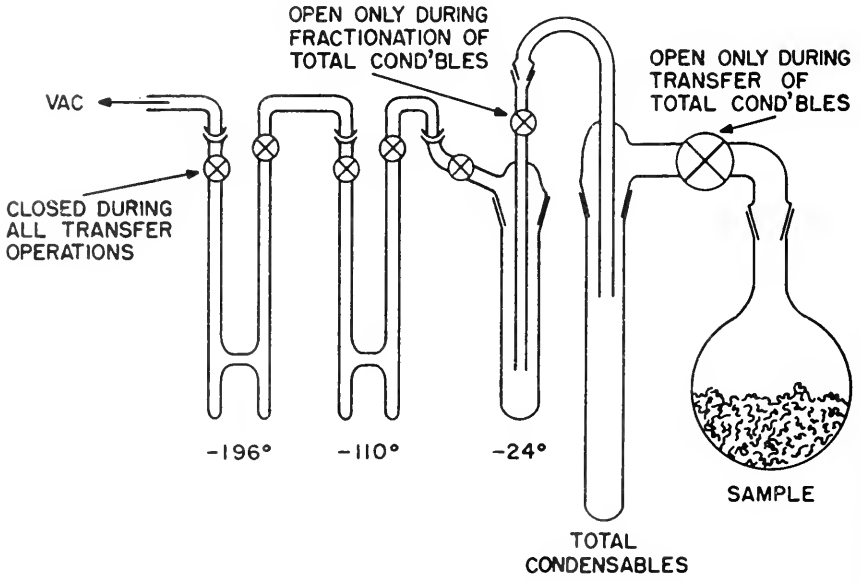


Figure 9. Low temperature distillation system.

bottom flask which will contain the raw meat sample. Connected and adjacent to it is a long annular trap for collecting the total condensables. Finally, connected to this trap is a smaller annular trap and two H-traps. These are attached to a pumping system.

To begin the concentration, the frozen meat was introduced into the liter flask. Air was pumped from the flask, while the meat was cooled with liquid nitrogen. The stopcock, following the long annual trap, was then closed and transfer was begun by warming the meat to room temperature and cooling the trap with liquid nitrogen. By experimenting, the most efficient period of this transfer was 18 hours.

After the period of total transfer, the meat was isolated from the total condensate by closing the stopcock. The total condensate subsequently was redistilled into the remaining three traps. These were cooled to minus 24°, minus 110°, and minus 196° C. The minus 24° C. trap held back most of the water during the distillations. The minus 110° C. trap served to retain the sulfides while passing the mercaptans. The minus 196° C. trap collected all of the very volatile condensables.

The period of this second separation was 2½ hours. The reason for this was to eliminate carry-over, from one trap to another, of frozen material, due to its solid phase vapor pressure. Work is presently continuing on the residue liquid phase of the total condensate which remained after redistillation and will be reported on at a later date.

Direct mass spectral analysis of the minus 24° liquid condensate showed that it consisted only of water. Thus, the compounds presented here will be only those which were present in the gas phase on the isolated minus 24°, minus 110°, and minus 196° C. traps after they were allowed to come to room temperature.

Mass spectrometric analyses along with pressure-volume measurements were made of these three separations. This was done to permit a quantitative presentation of the data. Only a small part of each separation was consumed in this operation so that a sufficient amount remained for further separation by gas chromatography.

The contents of each trap were further separated by isolative gas chromatography. The eluted fractions were condensed individually with liquid nitrogen. These cuts then were analyzed by a mass spectrometer.

All data are expressed in micromoles per kilogram of raw beef, and were calculated on a water-free basis although considerable quantities of that compound were encountered. Oxygen, nitrogen and argon resulting from occlusion are also omitted. The

relative concentration of each constituent is expressed to two figure accuracy. A series of sulfides and disulfides were found: dimethyl sulfide, methyl ethyl sulfide, methyl isopropyl sulfide, diisopropyl sulfide, dimethyl disulfide, diethyl disulfide, ethyl isopropyl disulfide, diisopropyl disulfide. The 0 M.R. column in the table represents the control or non-irradiated sample, while the next two columns indicate the compounds present after 2 and 4 megareps, respectively (table 3).

Table 3. Sulfides and disulfides found in meat vapor in micromoles per kilogram of beef

Compound	0 M.R.	2 M.R.	4 M.R.
Dimethyl Sulfide			1.8
Methyl Ethyl Sulfide			0.18
Methyl Isopropyl Sulfide			0.15
Diisopropyl Sulfide			0.053
Dimethyl Disulfide		0.11	0.11
Diethyl Disulfide		0.0059	0.42
Ethyl Isopropyl Disulfide			0.10
Diisopropyl Disulfide			0.19

Their identification after the mercaptans had been separated by distillation was a fairly simple matter by mass spectroscopy. In the absence of the mercaptans, both families of sulfides form a distinct series of peaks. Significant differences in the pattern of these peaks make specific identifications comparatively easy. It is interesting to note that almost every possible combination of the methyl, ethyl, and isopropyl radicals is included in the series of analyses.

The converse is true of the mercaptans which also formed an easily identifiable series of peaks. We can give no instrumental explanation for the decrease in concentration of ethyl, propyl, and butyl mercaptans from 2 MR to 4 MR, as shown in table 4.

Table 4. H_2S *and mercaptans found in meat vapor in micromoles per kilogram of beef*

Compound	0 M.R.	2 M.R.	4 M.R.
Hydrogen Sulfide	4.3	23.	39.6
Methyl Mercaptan	0.15	2.5	12.
Ethyl Mercaptan	0.28	1.1	0.082
Proypl Mercaptans		1.5	0.59
Butyl Mercaptans		0.18	0.11
Pentyl Mercaptans			0.045

However, with the exception of carbon dioxide, this is the only evidence of actual decrease in concentration of odor components with increase in radiation in any of our data.

Table 5. Carbonyl compounds found in meat vapor in micromoles per kilogram of beef

Compound	0 M.R.	2 M.R.	4 M.R.
Acetaldehyde		23.	170.
Pentanals		8.3	21.
Hexanals			6.5
Heptanals			1.1
Acrolein		8.8	23.
2-Met. Acrolein		0.25	3.3
Pentenals		Trace	Trace
Hexenals		1.8	4.2
Met. Et. Ketone		9.7	22.

Identification of the carbonyl compounds given in table 5 proved to be more of a problem. Unfortunately, the masses of various ionic species resulting from ionization of aldehydes, ketones and alkanes are very similar, thereby, making specific identification difficult. Here we used the rearrangement peaks peculiar to the aldehydes of which I have spoken earlier. By exploiting this behavior, we were able to obtain good indications of the type of aldehydes found. The most outstanding feature of this series of analyses is the prominence of the pentanals and the absence of propionaldehyde and butyraldehyde.

The same degree of certainty applies to the unsaturated aldehydes. Due to the lack of standard patterns for the pentenals, we can only indicate their presence from appearance of mass spectral peaks attributable to that type of compound. Although the presence of acetone was possible, methyl ethyl ketone was the only ketone which appeared present in any significant quantity.

The alkyl aromatics also formed a distinct series of peaks which facilitated their identification. Distinct pattern differences between single, double, and triple substituted benzenes made specific identifications fairly certain. With exception of trimethyl benzene, the general tendency of the benzenes appeared to be toward mono-substituted compounds. (See table 6).

Table 6. Alkyl aromatic compounds found in meat vapor in micromoles per kilogram of beef

Compound	0 M.R.	2 M.R.	4 M.R.
Benzene	(1)	0.74	4.9
Toluene	(1)	0.13	0.34
Ethyl Benzene	(1)	(1)	0.048
Isopropyl Benzene	(1)	(1)	0.082
Trimethyl Benzene	(1)	(1)	0.082
Butyl Benzene	(1)	(1)	0.034

[1] Not detectable.

Carbon dioxide was the most prominent of the volatile components detected with the exception of water (table 7). Inconsistent with most other compounds which showed increased concentration with increases of radiation dosage, we actually observed more carbon dioxide in vapor of unirradiated meat than in that from radiated meat. This change in carbon dioxide content possibly may be associated with the absence of amines from our investigation.

Table 7. Miscellaneous CPDS. found in meat vapor in micromoles per kilogram of beef

Compound	0 M.R.	2 M.R.	4 M.R.
Carbon Dioxide	4500.	1800.	1500.
Carbon Monoxide	110.	33.	65.
Methanol		5.0	16.
Ethanol		14.	39.
Ethylene		2.9	5.9
Propylene		2.9	4.4
Pyrrole		0.22	0.32
Pyridine		0.024	0.075
Aniline		0.007	0.098

It has been reported that amines in aqueous solution with carbon dioxide, as in meat, will form compounds of lowered volatility. With the production of amines by radiation, free carbon dioxide present in the meat would thus be consumed. (It is interesting to note that the compounds formed by this reaction are polar in nature and could contribute to flavor despite their low volatility.)

Identification of the 2 alcohols and 2 olefins shown in table 7 was certain, and the presence of higher homologues could not be established. Small unique peaks accounted for the identification of pyrrole, pyridine and aniline.

Due to overlapping mass spectral patterns, it is very possible that very small quantities of odorous compounds might be obscured by larger amounts of other material and thus be omitted from this report. Furthermore, this technique of analysis is restricted only to compounds with a fair degree of volatility. However, we believe that the study is representative of vapors over meat, and hence, also of its odor.

I would like to express my gratitude to the following colleagues who made most of this work possible. They are Messrs. Walter D. Niegisch, Eugene J. Levy, John T. Walsh, David C. O'Brien, Sidney Bresnick and Leonard F. Herk, Jr.

Discussion

MR. H. E. NEWLIN (Midwest Research Institute):

I would like to ask Dr. Stahl a question on his experiments on irradiated beef. Do you have any explanation or thoughts on the mechanism of formation of the compounds you found?

STAHL:

We have not really given it much thought, but in general it seems quite plain that a good many of these compounds come from the breakdown of the amino acids. I can see where the sulphur compounds come from but then, in order to be fair, I must say that we have not spent much time in looking for the answer to that question.

DR. S. PATTON (The Pennsylvania State University):

Dr. Stahl, how do you explain the presence of some of the compounds you have identified?

STAHL:

Most likely, most of them come from the recombination of fragments formed from the degradation products of the amino acids in the protein after irradiation with electrons.

III. FRUIT AND VEGETABLE FLAVORS

Techniques Employed in the Study of the Chemistry of Cabbage Flavor

TORSTEN HASSELSTROM

Chief, Organic Chemistry Branch, Pioneering Research Division
Quartermaster Research and Development Command

The Army Quartermaster is the largest consumer of food in the country. The problem is to provide the means of making concentrated products into foods of acceptable flavor, capable of keeping the soldier well satisfied. One problem is eating habits and the formulation, from dehydrated raw materials, of foods of a flavor we are used to at home. This is not an easy task because of the following reason. The great progress made in refrigeration has made the general public, including the potential soldier, unfamiliar with the pork- and meat-barrel of bygone days. With an abundance of fresh food available, dehydrated vegetables or food sterilized by radiation are not served in our homes. Nor has it been necessary anymore to keep up with the type of good cooking which in the past was necessary for providing savory, tasty food from not-too-fresh raw materials.

Much is known about the nutritive properties of foods (vitamins, calories, amino acids, fats, minerals, etc.). This information is adequate to assure a fairly good diet, regardless of whether the processed food be canned, frozen, or dehydrated. However, the information at hand on flavor is inadequate in many cases to assure acceptability. Our knowledge of the chemical composition of food flavors is inadequate, and for some products information on this point is meager, as for example, our common vegetables, beef, and pork.

The Pioneering Research Division has been assigned the study of the problem of food flavors, especially from the point of view of basic identification and characterization of active components.

In order to cover the necessary fields of chemistry, chromatography, spectroscopy, enzymology, and the art of sensory panel testing, we have employed not only our own staff but also the services of the Evans Research and Development Corporation.

Our objective is to study the chemistry of food flavors to provide fundamental information supporting the development of highly acceptable processed foods.

Modern warfare demands highly concentrated foods possessing high acceptability and maximum nutritional value. All foods, including dehydrated and irradiated foods, must have appetizing flavors to retain morale and stimulate adequate consumption under combat conditions.

Great emphasis in our studies has been given to the development of techniques for isolating and describing chemically and physically the individual compounds responsible for characteristic odor aromas and taste factors.

The processing of food is basically a stabilizing process. In most of the processing methods, the stabilizing is achieved by substantially destroying or inactivating all the enzymes, commonly done by blanching. The blanched food then is subjected to further processing, such as freezing, canning, or drying, to prevent or retard chemical changes and growth of bacteria on storage.

The result of the process is that the food no longer contains active enzymes. Depending upon the methods of processing, there will be other changes from the fresh food, some additive, some subtractive in nature. As far as fresh flavor is concerned, the effect is deleterious because many of the flavor components are volatile or heat labile. This is particularly true for the odor-flavor components. With respect to the taste-flavor components, many may be relatively non-volatile and heat stable, thus surviving the processing operation. In general, the processing more seriously affects the odor of the fresh food.

On the other hand, non-volatile, relatively heat stable compounds present in food will survive the process, and among these one can hope to find the flavor precursors. It follows, then, that the flavor precursors which survive processing represent a source of latent or potential flavor. If this potential flavor can be converted to actual flavor, a valuable improvement in the processed food will have been made. *We have found that the conversion of potential flavor in certain processed foods into actual flavor can be done by the addition of the proper enzymes (8).*

We differentiate between fresh flavor, altered flavors, and new flavors. A distinction is made between natural and off-flavors. To the latter category we add the concept of cooked flavors. What do we mean by off-flavors? Off-flavor may be defined as an aroma, odor, and taste we normally do not attribute to a certain food while it may normally occur in other foods.

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As an example of this concept, it might be mentioned that acetaldehyde is a normal constituent of apple flavor, but is definitely an off-flavor in butter where it sometimes occurs due to microbiological action *(10, 14)*. Hydrogen sulfide is an important component of beer flavor, where it is present below the threshold of olfactory sensitivity *(2)*.

The amount of flavor-odor constituents in our common foods is very small—1/100 to 1/100,000 of one percent *(10)*. In addition, many natural flavor-odor components are unstable and have a tendency to form off-flavors.

In our investigations we have made distinction between terpenes and non-terpenes as responsible for flavor-odor *(5)*. The reason for this is a chemical one. The terpenes have very much a chemistry of their own and, hence, are identified by rather specific methods not practical to use in other series. In addition, the terpenes are plant excretions and extracellular materials. We believe them to be waste products because they are pushed out from the living cell. The terpenes are water-free in their natural state and consist primarily of hydrocarbons in mixture with small amounts of oxygenated terpene materials. We believe, and have so postulated, that these oxygenated terpenes of an essential oil are responsible for its principal odor characteristics.

An illustrative example of that is pepper oil—the oil of natural black pepper. We have shown that most of the terpenes in pepper oil, about 1 percent of the peppercorn, are hydrocarbons but that a small quantity of oxygenated terpenes—about 4 percent of this oil—is responsible for the characteristic pepper aroma *(6)*.

Another example: About 25 years ago we investigated gum spirits of turpentine and found the characteristic aroma to be methylchavicol of anise flavor, present in amounts of about one-half percent *(7)*.

When terpenes are stored in the absence of light or air, they can be stored almost indefinitely without producing off-flavors; but they are unstable to water, particularly when acids are present, giving rise to a series of chain reactions, as, for example, those of hydration and dehydration. The cineol formation is noteworthy in the storing of citrus fruit juices, the sequence consisting of the pinenes and limonene which are hydrated to terpineol, which in turn is dehydrated to cineol.

The non-terpene flavor components are seldom of aromatic nature. They are mostly aliphatic esters, alcohols, aldehydes, ketones containing minute amounts of nitrogen or sulphur or nitrogen- and sulphur-containing compounds, accompanied by fats, sterols, and higher ketones. It would seem that the latter

compounds play the same role in the non-terpene series, as the terpene hydrocarbons for the oxygenated terpenes, namely, they are fixatives for the aroma *(5)*.

Many plants derive their odor from terpenes only. The flavor of our common vegetables is due to non-terpenes. Fruit and berry flavors may be a combination of both, as for example, citrus fruits and raspberries. The aroma and taste of meat and dairy products are due to non-terpene types of compounds.

We believe there is a great deal of difference between the aroma of a strawberry and those of the seeds of a strawberry. I would suspect that the terpene portion of strawberry flavor originates from its seeds because seeds, as we all know, often contain terpenes *(10)*. The meat of the strawberry is responsible for the fruity flavor.

In order to avoid pitfalls, investigative methods in flavor research have to be carefully selected, and procedures have to be modified to suit a particular food item. What is good for cabbage or strawberries may not necessarily apply to citrus fruit.

The direction of research on flavors at Pioneering Research Division of the Quartermaster Research and Development Command has been guided by the following new concepts and techniques, namely:

1. the role of enzymes in odor-flavor propagation;

2. the introduction of instrumentation; i.e., gas-liquid partition chromatography and mass spectrometry in odor identification;

3. the idea of flavor precursors and their separation, identification, and synthesis.

The first subject will be treated by Dr. Eric J. Hewitt of the Evans Research and Development Corporation, a research contractor of the Natick group.

Dr. William H. Stahl, chief of the Analytical Section of the Pioneering Research Division, will discuss instrumentation in flavor-odor identification.

Some years ago, we were given the task of investigating cabbage flavor. The reason for this was that the Army felt that the soldiers in the field should get a fresh salad in their diet. Fresh lettuce is not adaptable for this purpose, but it was thought that dehydrated cabbage could be used if the flavor could be improved. Therefore, cabbage became the first foodstuff investigated under this aspect of the flavor program. The flavor of cooked cabbage, as well as fresh cabbage, was studied in order to follow the results of dehydration.

Cooked Cabbage Flavor

When we started this work, there was nothing in the literature on the subject excepting for the fact that the flavor of cooked cabbage was described to be methyl mercaptan. This we found not to be the case.

We have identified compounds responsible for cooked cabbage odor. The compounds found were dimethyl disulfide and hydrogen sulfide. In addition, the precursor of dimethyl disulfide in the fresh vegetable has been determined. A paper on this subject is now in press *(4)*.

Development of quantitative and qualitative techniques for the identification of volatile sulfur compounds is a contribution to the problem of food flavor, for it has been shown by organoleptic techniques that such sulfur compounds can contribute to flavor even at levels below the threshold values characteristic of the compounds themselves. "Quantitation" of odor constituents is essential in exploring flavor changes in foods due to processing. Flavor acceptability depends greatly on concentration of the odor compounds.

Challenger and his coworkers used a train of absorption traps to investigate the products of microbiological methylation of sulfur compounds *(3)*. We were able to improve on their methods and adapt the technique to qualitative analysis of the sulfur odors of cooked cabbage.

The train of traps, illustrated in figure 1, serve to separate a mixture of hydrogen sulfides, mercaptans, sulfides, and disulfides.

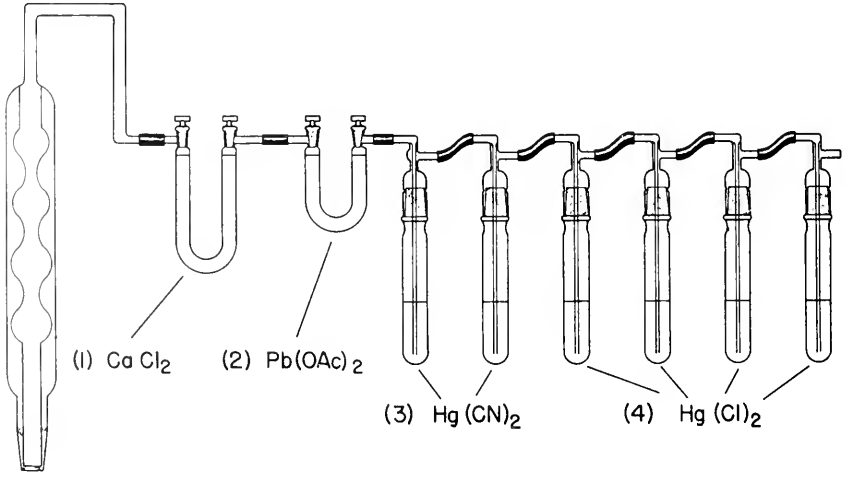


Figure 1. Absorption train for volatile sulfur compounds.

The modification of the Challenger train is the incorporation of traps (1) and (2) for the prior removal of hydrogen sulfide.

Cabbage is boiled under reflux and a stream of air or nitrogen is passed through the boiling slurry at a controlled rate of flow through the train. Absorption of the compounds is quantitative, except in the case of disulfides (table 1). If the gases in the air

Table 1. Qualitative absorption train

Trap	Substance absorbed	Reaction product
(1) $CaCl_2$ (S.)[1]	H_2O	
(2) $Pb(OAc)_2 \cdot 3(H_2O)$ (S.)[1]	H_2S	PbS
(3) $Hg(CN)_2$ (aq.)	RSH	$(RS)_2Hg$
(4) $Hg(Cl)_2$ (aq.)	RSR	$XRSR.\ yHgCl_2$
	RSSR	$RSHgCl.\ xHgCl_2$

[1] Traps (1) and (2) can be replaced by an acid solution of cadmium chloride.

stream are dried by calcium chloride, solid lead acetate will remove hydrogen sulfide as lead sulfide. Dry mercaptans do not react.

Aqueous mercuric chloride reacts with disulfides and monosulfides to give white precipitates of the composition shown. Identification of dimethyl disulfide is accomplished by acidification of the mercuric chloride precipitate and formation of the mercaptide in mercuric cyanide solution:

$$2CH_3SSCH_3 + (3 + x)\ HgCl_2 + 2H_2O \longrightarrow \underline{3CH_3SHgCl \cdot xHgCl_2} + CH_3SO_2H + 3HCl$$

chloromercury methylthiol mercurichloride methylsulfinic acid

$$CH_3SHgCl \cdot xHgCl_2 + HCl \longrightarrow CH_3SH + (x + 1)\ HgCl_2$$

$$2CH_3SH + Hg(CN)_2 \rightarrow (CH_3S)_2Hg + 2HCN$$

mercury dithiomethoxide

Table 2. Distribution of sulfur in cabbage

	g./100 g. of dry wt.	Reference
$(CH_3)_2S_2$ Sulfur	0.49	
H_2S Sulfur	0.01	
Nonvolatile Sulfur	0.30	*(1)*
Total Sulfur (Calculated)	0.80	
Total Sulfur	0.80	*(1)*

Analysis of the sulfur distribution in cabbage (table 2) shows that the volatile sulfur of cooked white cabbage can be accounted for by dimethyl disulfide and hydrogen sulfide. Results with dehydrated cabbage, red cabbage, broccoli, sauerkraut, and cauliflower yielded identical compounds. In no case was mercaptan detectable, even when aeration with nitrogen was used (table 3).

Table 3. Volatile sulfur in cabbage varieties

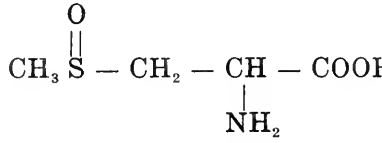
	Moisture, percent	Total sulfur	g./100 g. dry wt.	
			Disulfide sulfur	H_2S sulfur
White cabbage	91.9	1.0	0.054	0.003
Dehydrated cabbage		1.2	.030	.003
Red cabbage	93.5	0.7	.064	.008
Sauerkraut	91.4	0.7	.079	.010
Cauliflower	89.6	1.3	.183	.002
Broccoli	88.2	1.3	.150	.001

Use of a benzene trap (Segal and Starkey) *(12)* gives qualitative absorption of the disulfide and permits determination of disulfide by:

(1) Reduction with zinc and acetic acid.

(2) Volhard titration with silver nitrate and thiocyanate.

The precursor of dimethyl disulfide in cabbage was isolated by techniques of alcohol precipitation, ion exchange, and paper chromatography. It was identified as L-*S*-methylcysteine sulfoxide,



a sulfur containing amino acid recently discovered by Synge and Wood *(13)* in the dialyzate from cabbage juice.

L-*S*-methylcysteine sulfoxide was synthesized. In studying its decomposition under conditions similar to the production of dimethyl disulfide from cabbage, the following products were obtained: dimethyl disulfide, pyruvic acid, and ammonia.

In the course of the isolation of the precursor, it was shown that reaction with zinc and acetic acid produced methyl mercaptan, detectable as yellow silver mercaptide. The test turned out to be highly specific and sensitive, giving negative reactions with other sulfur-containing amino acids, known to be in cabbage, such as methionine, methionine sulfoxide, and cysteine.

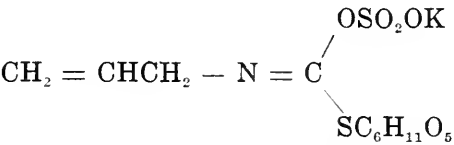
In summary, techniques have been devised for quantitative and qualitative identification of volatile divalent sulfur compounds. These methods have been shown applicable to problems in flavor

identification. With suitable modification (by adopting more sensitive colorimetric reactions for determining mercaptans and hydrogen sulfide), they can be used to detect differences in parts per billion.

Fresh Cabbage Flavor

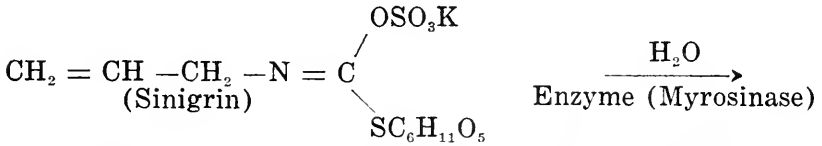
Research in the field of flavor precursors is based on the premise that the characteristic flavor-odor of vegetables and fruits is due to the volatile odor of compounds of relatively low molecular weight which have been formed from higher molecular weight compounds by enzymatic action.

Using a system of paper chromatographic analysis devised by Schultz in 1952, the Evans Research and Development Corporation corroborated Schultz's identification of sinigrin, a thioglucoside

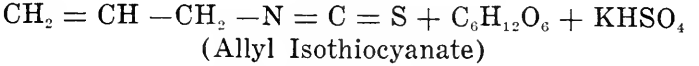


in white cabbage seed, and suggested the possibility of a second similar compound in white cabbage leaf, in addition to sinigrin *(11)*. Jensen *(9)* in 1954, also reported a trace of such a chemical in white cabbage seed.

The significance of the detection of thioglucosides in cabbage is the fact that isothiocyanates are produced from them by the action of enzymes in a 2-step process.



(Sinigrin) (Enzyme (Myrosinase))

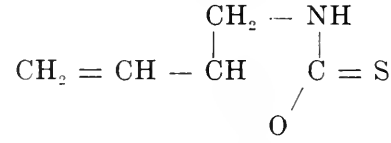


(Allyl Isothiocyanate)

Isothiocyanates are pungent materials which contribute to flavor, even when present in minute amounts.

For instance, in the case of the action of the enzyme complex, myrosinase, obtained from mustard seed, or sinigrin, it is believed that the sulfatase moiety causes the hydrolysis of the sulfate ion and that the thioglucosidase portion hydrolyzes the linkage of glucose with the isothiocyanate component. The products of the reaction are allyl isothiocyanate, the well-known oil of mustard, a hexose (glucose), and a salt, potassium bisulfate.

Astwood et al. *(1)* have found that isothiocyanates are the precursors for thioxazolidone, since they were able to isolate L-5-vinyl-2-thiooxazolidone in cabbage seed:



With this background, we are investigating the characteristic aroma factors in fresh cabbage. The procedure employed is the following:

One hundred pounds of white cabbage was shredded and left standing for 24 hours at room temperature to assure maximum activity of the myrosinase enzyme complex. The mixture then was extracted with isopentane or hexane. An aliquot of the extract was dried and treated with ammonia in order to convert the isothiocyanates into the corresponding solid thioureas.

By means of paper chromatography, using chloroform saturated with water, three spots were obtained. One of these was the thiourea of allylisothiocyanate, mustard oil, as the main product. This result was also confirmed by chemical identification, elementary analysis, and the mixed melting point test with an authentic sample. Presently, work is in progress for possibly identifying the two other isothiocyanates.

We have found that minute quantities of isothiocyanates can also be identified as thiosemicarbazides when solutions containing isothiocyanates are reacted with hydrazine hydrate. This is of interest because the thiosemicarbazides, in turn, react with carbonyl compounds, by which means the molecular weight—and consequently the yield of derivative—can be increased, making identification more convenient.

These are our results to date. We ask ourselves what good is this information for the improvement of the unit processes for dehydration of cabbage? The answer may be formulated as follows:

Sinigrin, the precursor for allylisothiocyanate—the main flavor characteristic for fresh cabbage—is stable at 100° C., but water soluble. The L-*S*-methylcysteine sulfoxide—the precursor for cooked flavor—is quite water soluble and not stable in cabbage at temperatures above 50° C. This means that a minimum of water should be used in washing of the cabbage prior to dehydration, which should be carried out at a temperature below 50° C. In case this is impractical, then synthetic L-*S*-methylcysteine sulfoxide and puruvic acid should be added. If coleslaw is the desired

end-item, the flavor of fresh cabbage is restored by addition of the myrosinase enzyme mixture.

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Discussion

HAAGEN-SMIT:

I have a question about definition. If I heard correctly, you divide the flavor components into terpenes and other compounds, is that right?

HASSELSTROM:

I classify them into terpenes and non-terpenes because of the fact it is so convenient to do so. The terpenes are compounds which are collected outside of the living cell.

HAAGEN-SMIT:

I was wondering if you would not then get into trouble in connection with the gases?

HASSELSTROM:

Yes, but then I leave them together.

HAAGEN-SMIT:

I think that we always get into trouble with definitions anyway. I would like to make a remark about the compounds from the cabbage. We have synthesized about 6 different compounds and we have found that at least 1 of them was quite remarkably similar to cabbage flavor.

HASSELSTROM:

Well, I am not at all surprised, because if you will look closely at your compound you may find that it originated from some of the things that I have described.

Flavor Propagation Through Enzymatic Action

by

E. J. HEWITT, D. A. M. MACKAY AND K. S. KONIGSBACHER

Evans Research and Development Corporation

and

TORSTEN HASSELSTROM

U. S. Army Quartermaster Research and Development Command

The majority of this research has been reported previously at the Sixteenth Annual Meeting of the Institute of Food Technologists in St. Louis on 13 June 1956. It also has been published in the October 1956 issue of *Food Technology*.

This work was carried out under contract to the Quartermaster Research and Development Command, Natick, Massachusetts. The overall purpose of the program was to improve the flavor of processed foods, particularly dehydrated foods, which are important to the armed services.

Industrial food processors also are concerned with problems of flavor. Food processing often alters or destroys the fresh natural flavor of fruits, vegetables, and other natural food products. The purpose of this paper is to show that fresh natural flavors may be restored in processed foods by enzyme action.

The characteristic flavors, that is, the odor and taste of fresh fruits, vegetables, and other fresh animal and plant products are caused by chemical compounds which have been produced by normal metabolic processes. Presumably, such flavors have been formed from other chemical substances (which we call flavor precursors). These flavor precursors have themselves been formed from their precursors, making it possible to picture the formation of a series of chemical substances in a chain of reac-

tions which might begin with carbon dioxide, sunlight, water, and minerals.

These chemical changes are brought about by the catalytic effect of enzymes, which are naturally occurring proteinaceous materials found in the organism. These enzymes are often highly specific for bringing about the chemical changes in each step of the complicated process whereby the flavor precursor is built up and then converted into the flavor itself. The actual flavor may be made up of one or many natural flavors. Each natural flavor may require for its biosynthesis a flavor precursor and an enzyme system. Complex natural flavors might, therefore, require complicated biosynthetic processes containing many precursors and enzymes.

The enzymes are thermal-labile and tend to lose their ability to catalyze chemical reactions when heated. They also can be inactivated by other means, such as irradiation or "poisoning" with certain chemical compounds.

The processing of food is basically a stabilizing process. Most processing methods stabilize the food by substantially destroying or inactivating the enzymes. This is most commonly done by blanching, a process in which the food is subjected to wet heat, usually hot water or steam. The blanched food then is processed further, by freezing, canning, or dehydration, to prevent or retard chemical changes and growth of bacteria on storage.

The result of the process is that the food no longer contains active enzymes. Depending upon the methods of processing, there will be other changes from the fresh food, some additive and some substractive in nature. Processing destroys or alters part of the fresh flavor, because many of the flavor components are volatile or heat-labile. Thus, in the study of food products, it is important to determine what part the flavors of the fresh food (that is, the enzymatically produced flavors) play in the acceptability of the food or food products as presented to the consumer. The flavor of the processed food may be composed of the fresh flavor which has persisted through processing, the flavors which come from conversion of the fresh flavors originally present, and the flavors which arise during processing.

Non-volatile, relatively heat-stable compounds present in the food will survive processing, and among these appear to be the flavor precursors. These surviving flavor precursors then represent a source of latent or potential flavor. If this potential flavor can be converted into actual flavor, a valuable improvement in the processed food will have been made. We have found that potential flavor in processed foods can be converted into actual

flavor by adding the proper enzymes. This is shown in figure 1, and illustrated graphically in figure 2.

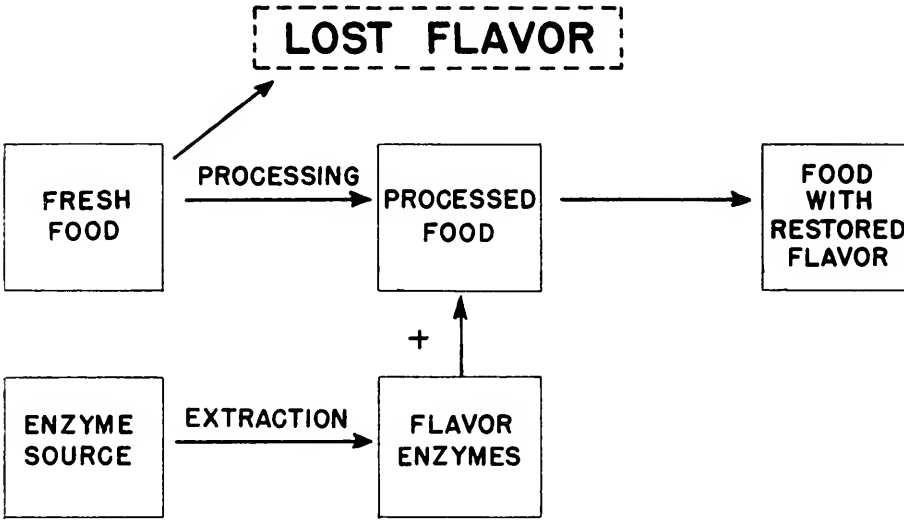

Figure 1. Restoration of natural flavor to processed foods.

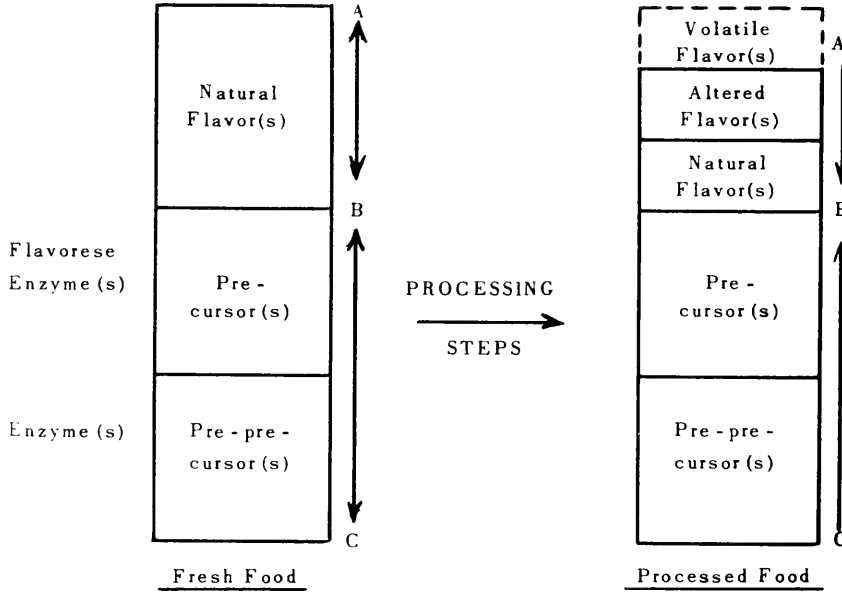

Figure 2. Barographs illustrating survival of flavor precursors and pre-precursors in conventional heat-processing.

In figure 2, the first barograph represents the composition of a fresh food before it is processed. For purposes of discussion, this food contains three important constituents: the natural flavors, the flavor precursors including pre-precursors, and the flavor-producing enzymes which convert the precursors into flavors.

The natural flavors in the fresh food might be called actual flavor and their amount indicated by the line A–B. The flavor precursors plus pre-precursors in the fresh food might be called potential flavor and the amount indicated by the line B–C. The relative amounts of actual flavor and potential flavor in the barograph are arbitrary.

We have called the enzymes, which convert flavor precursors into flavors, flavorese enzymes. The suffix, —ese, indicates that the enzyme or enzymes are concerned with the formation of flavors. The more commonly used suffix, —ase, refers to the nature of the substrate upon which the enzyme acts. In addition to the flavorese enzymes, there must be other enzymes in the fresh food which convert pre-precursors into precursors.

Heat processing causes a loss of some volatile flavor components and the alteration of some of the heat-labile flavor components. Some of the natural flavors, however, probably survive the processing. The amount of actual flavor after processing is indicated by the line A–B. This is less than that originally present in the fresh food. Under severe heat processing, such as in dehydration, this loss is often very great, and only a small fraction of the actual flavor may survive. Since enzymes are relatively heat-labile, essentially no enzymes remain after processing. The flavor precursors have the greatest chance for survival of any of the flavor contributing factors because they apparently are non-volatile and heat-stable. According to this barograph, all the flavor precursors and pre-precursors have survived the processing step, since line B–C is the same length for both the fresh and processed food.

The first barograph in figure 3 again represents the processed food. The actual flavor is represented by line A–B, the potential flavor is indicated by line B–C, and there are no enzymes present. The second barograph represents the processed food after the addition of flavorese enzymes. All the flavor precursors have been converted into natural flavors. The actual flavor, line A–B, is a combination of those natural flavors which have remained during processing, altered flavors which resulted from the processing, and natural flavors resulting from enzyme action on the flavor precursor. The potential flavor remaining has been reduced to the line B–C.

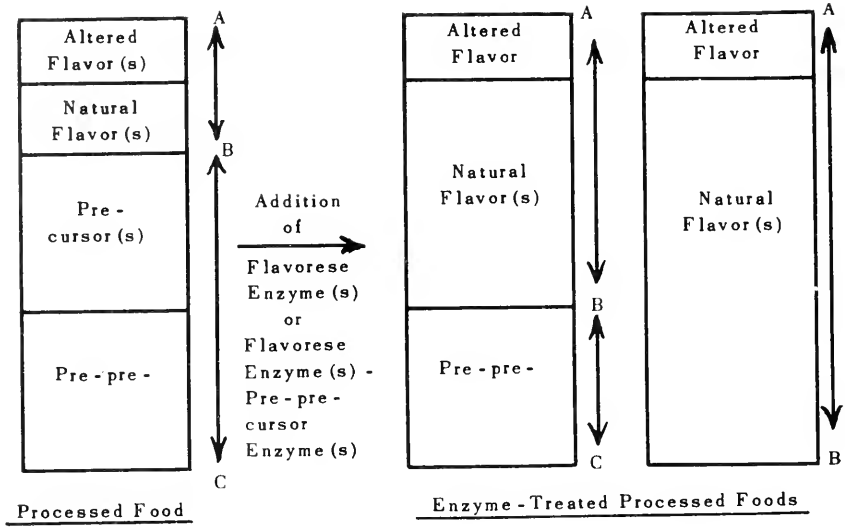

Figure 3. Barographs illustrating action of enzymes on flavor precursors and pre-precursors in processed food.

In the third barograph, a combination of flavorese and pre-precursor enzymes converts all the potential flavor into actual flavor as represented by A–B. The potential flavor, line B–C, has disappeared completely. Therefore, to obtain the maximum amount of actual flavor in a processed food, enzymes should be added to the processed food which convert as much as possible of the potential flavor into actual flavor.

The flavorese enzymes, necessary for the conversion of the flavor potential or flavor precursors to the flavors, can be obtained from the fresh biological material before processing, or from a biologically related source by one of the usual processes for the preparation of enzymes. Our studies have indicated that biologically related materials have related enzyme systems. This made it easier to select the raw material for the enzyme source.

It is better to start with a rich natural source of enzyme. However, it is not necessary to obtain an absolutely pure sample of flavorese enzyme, since a relatively crude enzyme preparation will be effective if it contains a high enough concentration of the desired enzyme(s).

These enzymes presumably would be added to the processed food before it reaches the consumer. If, however, the crude enzyme preparation contains some unwanted enzymes which are not

concerned in the formation of fresh flavor and which might cause degradative changes in the food in storage, it would be better to add the flavorese enzyme just before the food is prepared for consumption. Active enzyme preparations can convert the potential flavor into actual flavor very quickly. This may be because heat processing has increased the permeability of the cell walls of the food, making it possible for rapid diffusion and intermixing of the enzyme and precursor. Enzyme preparations which do not contain deleterious enzymes can be added to the food at any time or at any stage in processing, provided no processing steps follow which would inactivate the enzyme.

Our initial experimental work included studies on cabbage, watercress, and mustard, which are all members of the Cruciferae family. Some of the more important members of this biological family are shown in table 1.

Table 1. The more important vegetables in the Cruciferae family

Turnip	Rutabaga
Cabbage	Mustard (black and white)
Kale	Cauliflower
Watercress	Brussel Sprouts
Horseradish	Radish
Charlock	Candytuft
Rape	Pennycress

In one experiment on watercress, fresh watercress was blanched in steam and dehydrated in an oven at about 60° C. for 3 hours. The dehydrated material was quite flavorless and had none of the characteristic taste and smell of watercress. When water was added to this material, no change was observed. The reconstituted watercress smelled and tasted like hay. However, when a tasteless, odorless, enzyme preparation from white mustard seed was added to the dehydrated watercress in water, the typical odor and taste of watercress were regained within minutes.

Dehydrated cabbage also reacted with white mustard seed enzyme to give an increase in flavor. Enzyme preparations from black mustard seeds also are effective. This is illustrated in figure 4.

Mustard seed contains the enzyme, myrosinase. This enzyme complex is believed to be made up of two enzymes, a sulfatase and a thioglucosidase. When it reacts with a thioglucoside, such as sinigrin, which is present in black mustard seed, allyl isothiocyanate is formed.

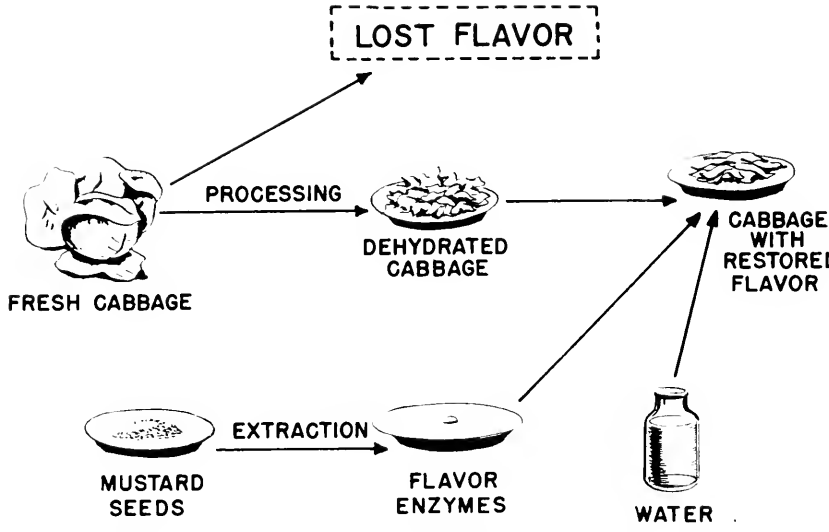

Figure 4. Restoration of natural flavor to dehydrated cabbage.

$$CH_2=CH-CH_2-N=C\begin{matrix}OSO_3K\\ \diagup\\ \diagdown\\ SC_6H_{11}O_5\end{matrix} \xrightarrow[\text{Enzyme (Myrosinase)}]{H_2O} CH_2=CH-CH_2-N=C=S + C_6H_{12}O_6 + KHSO_4$$

(Sinigrin) (Allyl Isothiocyanate)

Figure 5. Formation of allyl isothiocyanate (mustard oil) by means of enzyme action.

The products of the reaction are the mustard oil, allyl isothiocyanate, plus glucose and a salt.

Ettlinger *(1)* recently has proposed a different structure for sinigrin:

$$R-\underset{\underset{N-OSO_3}{\|}}{C}-SC_6H_{11}O_5$$

There are probably many naturally occurring compounds related to sinigrin. One is the thioglucoside, sinalbin, which occurs in white mustard seed. The action of myrosinase on sinalbin results in the formation of p-hydroxybenzylisothiocyanate.

The enrichment of fresh natural flavor, which occurred when flavorese enzymes were added to dehydrated watercress and dehydrated cabbage, is possible with many other processed fruits and vegetables. Some of these are shown in table 2. These foods

were processed in various ways. Some were canned, some frozen, and some dehydrated.

Table 2. Processed foods improved in flavor by addition of enzymes

Celery	Tomatoes
Parsley	Bananas
Spinach	Oranges
Carrots	Strawberries
Onions	Pineapple
Milk	

To test the effect of the appropriate flavorese enzyme on the flavor of these processed foods, each was divided into two samples. The enzyme was added to one sample, and the other untreated sample was retained as a control. After allowing a short time for the reaction to occur, the flavor or odor of the samples was compared by a small, trained group. In all cases there was a flavor improvement with a regain of fresh flavor, but, in some cases, the effect on flavor was greater than in others.

To obtain the maximum yield of flavor regain, there must be adequate concentrations of the required enzyme and of the precursor to react within the allotted time. Water also is required for the reaction; pH and temperature are also important.

To substantiate further the theory that the regeneration of flavor is an enzymatic phenomenon, several experiments were conducted. These included both heat inactivation tests and pH activity tests on onion and watercress flavorese enzymes.

In the heat inactivation tests, the watercress enzyme was heated for 2 minutes at 100° C. at pH 6.0. The onion enzyme was heated for 2 and 3 minutes at 100° C. at pH 5.6. The heated enzymes then were assayed on a dehydrated onion substrate and a heat-treated watercress substrate. No watercress or onion flavor developed when the heated enzymes were used, while the control (unheated) enzymes produced strong odors. Since these materials can be inactivated by heating for 2 minutes at 100° C., this substantiates further the theory that they are enzymes.

In the pH activity tests, the onion and watercress substrates were adjusted to various pH's. Both onion and watercress enzymes then were added to the substrate. The results, which are shown in table 3, show that the optimum pH range for these enzymes is 5 to 7.

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Table 3. pH Activity ranges for onion and watercress enzymes

Onion		Watercress	
pH of substrate	Flavor	pH of substrate	Flavor
2.0	None.	2.0	None.
3.0	Weak onion.	3.0	Very weak watercress.
4.0	Medium onion.	4.0	Weak watercress.
5.0	Strong onion.	5.0	Strong watercress.
6.0	Very strong onion.	6.0	Strong watercress.
7.0	Very strong onion.	7.0	Strong watercress.
8.0	Weak onion.	8.0	Medium watercress.
9.0	Decayed onion.	9.0	Weak watercress.
10.0	Decayed onion.	10.0	Weak watercress.
11.0	Decayed onion.	11.0	Very weak watercress.
12.0	Decayed onion.	12.0	None.

Since the presence of the precursor is required in the processed food for the effect of enzyme addition to be observed, it is clear that for the maximum effect the conditions of processing must be such that the precursor is preserved throughout processing to the greatest possible extent. Thus it may be more desirable to plan the processing treatment with the intent of securing the maximum precursor survival rather than to plan for maximum flavor survival.

This is illustrated by the case of watercress. The enzyme in watercress responsible for converting the precursor to the flavor is very heat-labile, and the conditions of dehydration (60° C.) are sufficient to inactivate it. Thus, when watercress is dehydrated without previous blanching, the product is flavorless, and rehydration brings back no flavor.

Dehydrated unblanched watercress, dehydrated steam-blanched watercress, and dehydrated boiling water-blanched watercress are all bland and flavorless. However, when the white mustard enzyme preparation is added to the water of rehydration, a marked difference appears. The unblanched quickly regains its strong taste and smell. The steam-blanched has, by comparison, a moderately strong regain, and the boiling water-blanched has only a weak regain of characteristic flavor. Moreover, the water used for the hot water blanch is quite flavorless, but, when treated with the enzyme, it soon develops a strong watercress flavor. This proves that hot water-blanching has leached out the precursor and reduced the potential flavor of the processed watercress. It also shows that steam blanching is to be preferred in this case, although even this leads to slight losses.

To apply this process to any particular processed food, studies would be required to see whether the precursor survives process-

ing. If the process involves blanching, studies would have to be made on the various methods of blanching. There are three common commercial methods of blanching: steam at atmospheric pressure, steam under pressure, and hot water. With these three methods, many combinations of temperature and amount of water are possible. The flavor precursors in foods may show varying degrees of heat stability and water solubility. If so, commercial application of this process would require a study of these properties for any particular food. The hot water blanch could be modified to reduce losses with very water-soluble precursors by repeated use of the same blanch water. This sometimes is referred to as serial blanching. Steam-blanching, particularly under pressure, also might avoid the loss of flavor precursors which are very water-soluble.

To apply this technique to any processed food, studies would have to be made to find a satisfactory source of enzyme. The dozen or so industrial enzymes which we have tried (amylase, cellulase, invertase, maltase, inulase, glutaminase, catalase, nuclease, lactase, lipase, glucosidase, protease, and pectinase) have not proven useful. This is not surprising because of the specificity of enzymes and the limited number available commercially. There are probably hundreds of enzymes known to science which are not available commercially.

Enzymes occur in many kinds of biological materials. Methods used for concentration will depend upon the nature of the starting material. In general, the process would consist of an extraction and then a precipitation. The final preparation should have adequate stability and activity. The enzyme probably would be more convenient to handle and more stable if converted into the form of a powder or solid. Some enzymes are much more stable than others, and their stability sometimes depends upon their purity. All these factors would have to be considered in commercial practice. It would be important commercially to find as rich a natural source of enzyme as is possible. The purification of enzymes is a difficult task which usually is accomplished by 1 of the following 5 methods:

1. fractional precipitation with solvents or salt or both;
2. adsorption and then elution from the adsorbents;
3. crystallization brought about by cooling or salting out;
4. purification by ultracentrifugation;
5. purification by electrophoresis.

The last three methods would be most difficult on a commercial scale.

To illustrate flavor propagation through enzymatic action to representatives of the Government and industry, demonstrations were set up. In these demonstrations, the representatives, both food experts and nontechnical people, acted as judges of the enzymatically enhanced food flavors.

Commercially dehydrated cabbage, commercially dehydrated carrots, canned onions, laboratory dehydrated watercress, laboratory dehydrated horseradish, canned tomato juice, and commercial dried skim milk solids were used, as well as the following flavor precursor concentrates: cabbage, carrot, tomato, watercress, onion, and rose. Selection of test materials for the demonstration was based on previous tests and on the commercial importance of the material. Some foods were chosen where the enzymatic flavor enhancement is startling, and others where the enhancement is much less perceptible.

Before the start of a demonstration, it was explained to the participants that they were participating in a demonstration and not in a scientific test run according to sensory techniques. They were told also that flavorese enzymes enhanced fresh flavor which they might not be accustomed to (such as in tomato juice), and that the differences in the case of bland foods might be small.

In the demonstration, two series of materials were presented to the participants. The first series consisted of processed foods. Some of these foods did not contain flavor precursors, and, therefore, we added precursor solutions to them. (As a matter of fact, we have found that the flavor precursors often do not survive commercial processing.) The second series consisted of purified flavor precursor concentrates. Two sets of samples were presented in each series: one with the flavorese enzyme added and the other without the enzyme. Both the purified flavor precursor concentrates and the enzymes are essentially flavorless until mixed. Because of the time required to prepare the samples for the demonstration, the enzymes were added beforehand.

A photograph of a typical demonstration setup is shown in figure 6.

The solid test foods were placed on a table in shallow covered dishes together with labeled shot glasses. Each participant was instructed to ladle as much of the test material into the shot glass as he desired. Liquid foods were presented in individual brandy snifters. The flavor precursor concentrates were placed in covered brandy snifters, and the participants were instructed to pour as much as they wanted into small paper cups provided for that purpose. All samples were clearly labeled.

Each participant also was given a questionnaire, shown in figure 7, to fill out.

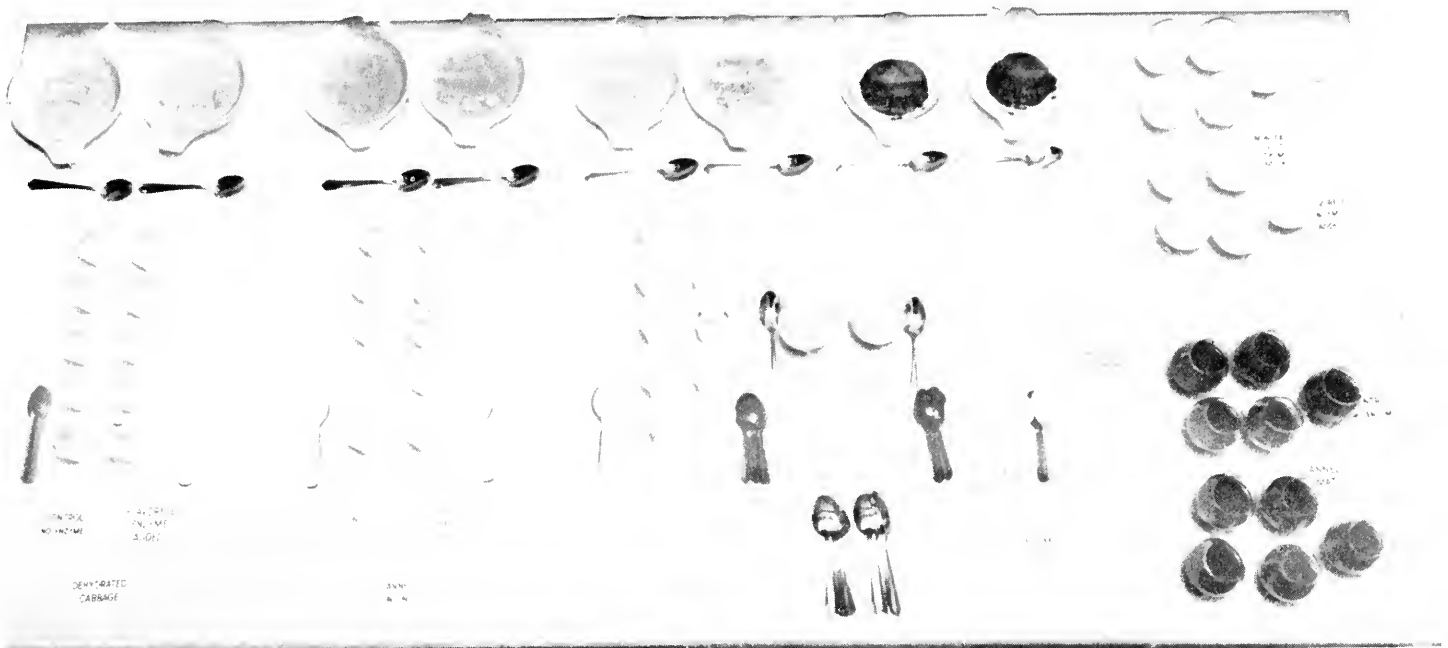

Figure 6. Demonstration setup used to illustrate flavor propagation through enzymatic action.

DEMONSTRATION OF FLAVORESE ENZYME PROPAGATION

For this demonstration, the following food products have been treated with odorless flavorese enzymes: dehydrated cabbage, dehydrated carrots, canned onions, dehydrated watercress, dehydrated horseradish, canned tomato juice, and reconstituted dried skim milk solids. Untreated controls are available for comparison.

The following flavor precursor concentrates have been prepared and treated with flavorese enzymes: cabbage, carrot, tomato, watercress, onion, and rose. Untreated controls are also available for comparison.

We have prepared the following table for your convenience. Won't you jot down your observations on which sample smells and tastes more like the *fresh* product.

0 = no difference
+ = slight preference
++ = distinct preference
+++ = strong preference
++++ = very strong preference

Food product	Control (no enzyme)	Enzyme treated	Comments
Dehydrated Cabbage			
Dehydrated Carrots			
Canned Onions			
Dehydrated Watercress			
Dehydrated Horseradish			
Canned Tomato Juice			
Reconstituted Dried Skim Milk Solids			
Cabbage Flavor Precursor Concentrate			
Carrot Flavor Precursor Concentrate			
Tomato Flavor Precursor Concentrate			
Watercress Flavor Precursor Concentrate			
Onion Flavor Precursor Concentrate			
Rose Flavor Precursor Concentrate			

Figure 7. Questionnaire used in demonstrations of enzymatically enhanced food flavors.

After the participants had filled their individual receptacles, they sat down in the booths provided to judge the samples undisturbed. Ice water and crackers were available for the waiting period between samples.

The data obtained from the questionnaires of a group of Government representatives and two groups of industrial representatives are summarized in table 4. A total of 242 judgments was

Table 4. Enzymatic flavor enhancement demonstration ratings

Material	Preference for untreated sample		No difference	Preference for treated sample			
	Distinct	Slight		Slight	Distinct	Strong	Very strong
Dehydrated cabbage	1	1		4	10	3	1
Dehydrated carrots		1	4	6	7	2	
Canned onions				4	13	2	
Dehydrated watercress		1		1	6	4	8
Dehydrated horseradish					4	2	11
Canned tomato juice	1	2	4	1	8	4	
Dry skim milk	2		3	5	9	1	
Cabbage precursor		1	2	2	8	6	
Carrot precursor		3	2	3	8	2	1
Tomato precursor	1	4	1	1	12	2	
Watercress precursor		1		2	8	5	3
Onion precursor				2	5	11	1
Rose precursor			3	3	2	1	
Total	5	14	19	34	100	45	25
Percentage	2.1	5.8	7.8	14.1	41.3	18.6	10.3

obtained. These data show that a difference was found in 92.2 percent of the cases. Of this total, the treated samples were preferred 84.3 percent of the time; the untreated controls, 7.9 percent. The preference ratings for the treated samples can be broken down as follows: 14.1 percent showed a slight preference, 41.3 percent a distinct preference, 18.6 percent a strong preference, and 10.3 percent a very strong preference.

As might be suspected, there was considerable variation in scoring for the different test materials. A difference between the treated and untreated samples was found in 100 percent of the judgments on dehydrated cabbage, canned onions, dehydrated watercress, dehydrated horseradish, watercress precursor, and onion precursor. With tomato precursor, a difference was found in 95 percent of the cases; with cabbage and carrot precursors, in 90 percent of the judgments; with dried skim milk, in 85 percent; with dehydrated carrots and canned tomato juice, in 80 percent; and with rose precursor in 6 percent of the cases.

A similar distribution was found for the preference ratings. Every judgment indicated at least a distinct preference for the treated dehydrated horseradish. For the other test materials, at least a distinct difference was noted in the following percentages of ratings: 70 percent for dehydrated cabbage, 45 percent for dehydrated carrots (75 percent total preference for treated sample), 79 percent for canned onions, 90 percent for dehydrated watercress, 60 percent for canned tomato juice, 50 percent for dried skim milk (75 percent total preference for treated sample), 74 percent for cabbage precursor, 58 percent for carrot precursor, 67 percent for tomato precursor, 84 percent for watercress precursor, 90 percent for onion precursor, and 33 percent for rose precursor (67 percent total preference for treated sample).

Although it is obvious that an understanding of food technology and enzyme chemistry is required for adopting this process, indications are that it is well worthwhile to investigate the introduction of enzymes into food processing in view of the improvement possible in the fresh flavor of many processed foods.

Literature Cited

1. Ettlinger, M. G., and Lundeen, A. J. *J. Am. Chem. Soc., 78,* 4172 (1956).

Discussion

DR. J. G. WOODROOF (Georgia Agricultural Experiment Station):

I don't understand how you isolated your enzymes and what was the source for your enzymes.

HEWITT:

Well, the isolation of an enzyme is a big problem. What we did in most cases was to get an enzyme preparation that would give activity or that would give an effect within a few minutes or an hour or so at the most.

In connection with the source of material, we took several things into consideration. One of them was whether it was easy to work up the material and whether or not it was a very rich source of very active enzymes. In general, however, we had two guiding principles. We either took it from whatever food substance we were considering or from a botanically related food substance.

McINTIRE:

What was the maximum temperature, generally, to which you could subject them without doing any damage?

HEWITT:

The most usual temperature at which enzyme activity starts to fall off is at 50° C.

MR. A. H. MICHEELS (Polak's Frutal Works, Inc.):

In that connection, then, how do you then prepare the precursor from tomato juice. Is it all the soluble material or is there further purification?

HEWITT:

In the plant cell you have both a precursor and an enzyme. In preparation of the enzyme or precursor, the enzyme could cause changes to occur in the precursor. Therefore, you must, during preparation, separate the precursor from the enzyme immediately or you will have no precursor left. The easy way to do that is by heat, and then you have a solution with the enzyme inactivated. It is difficult to state an overall procedure in detail because there are probably hundreds of different types of precursors, and conditions have to be altered to fit the individual case.

MR. M. WEBER (Pabst Research Laboratories):

Do you have a procedure to detect the presence of the enzyme?

HEWITT:

This might be done, but the procedure would depend upon which enzyme you are after. The best sensing mechanism for the presence of these "flavorese" enzymes is your own sense of taste or smell.

DR. A. D. CAMPBELL (Standard Brands, Inc.):

Has any work been done relative to the development of flavor in potatoes?

HEWITT:

We have not done anything on this. We do not know what effect dehydration would have on the flavor.

McINTIRE:

When you ran the milk flavor test, I understood that you added the enzyme sometime before the milk was tasted. Was the control milk made up at the same time?

HEWITT:

Yes, it was made up at the same time.

Chemistry of the Volatile Citrus Flavors

W. L. STANLEY

Fruit and Vegetable Chemistry Laboratory
Western Utilization Research Branch
Agricultural Research Service
United States Department of Agriculture

The volatile citrus flavors belong primarily to the chemical family of terpenes, the simplest of which is the 5-carbon hydrocarbon isoprene. The 10-carbon terpene hydrocarbon D-limonene represents over 85 percent of lemon oil and over 90 percent of orange oil. The terpenes are high-boiling, chemically unsaturated, branched-chain compounds representing varying degrees of condensation of isoprene units and possessing various cyclic and acyclic structures and degrees of oxidation. These compounds undergo structural rearrangement and hydration reactions under the influence of acids and are rapidly oxidized by air or dissolved oxygen. The rates at which these transformations take place are markedly affected by heat, which explains the improvement in flavor obtained in low-temperature *versus* high-temperature evaporation processes used in preparing citrus concentrates.

Volatile flavors from citrus juices are low in water and alcohol solubility, which accounts for the fact that homogeneous essences cannot be prepared from citrus juices as has been done by the Eastern Utilization Research Branch with apple juice. In contrast, condensates from citrus juices separate into two phases, an oil phase and a water phase, the former containing almost all of the characteristic citrus flavor. The water phase is practically worthless for use in flavor fortification.

Histological examination of sections of the juice sacs of citrus fruits has revealed the presence of minute deposits of oil, wax, and granular matter *(1)*. This oily material, however, makes only a minor contribution to the total recoverable oils in commercially-reamed juices. Compression of the peel in the extraction of juice releases oil which is incorporated in the juice. Comparisons made of juice from carefully hand-peeled oranges with hand-reamed orange juice have shown that over 50 percent of the oils in the hand-reamed juice originated in the peel *(9)*. It has been estimated that over 80 percent of the volatile oils in commercially-reamed, single-strength orange juice, and over 93 percent of the oils in commercial frozen orange juice concentrate may originate in the peel.

The citrus peel oils are deposited in ductless glands located in the colored outer layer (flavedo) of the peel. These oils are re-

covered commercially as water emulsions by squeezing the spent peel, after extraction of the juice, in screw-driven presses, passing the peel through grooved rollers, or by rasping the surface of whole fruit with abrasive rollers or compressing the whole fruit during the juicing operation. The oils are separated from the water emulsions in high speed centrifuges. Their main uses are in flavoring bakery goods, candies, and soft drinks, and for perfuming soaps and other cosmetic preparations. It has been demonstrated at the Pasadena Laboratory that cold-pressed peel oils can be used in the flavor fortification of 4-fold frozen orange juice concentrate *(9)*. The current practice in preparing frozen concentrates commercially, however, is to evaporate the juice to a 5-fold concentration and then to restore flavor by diluting to 4-fold concentration with freshly reamed, single-strength "cut-back" juice. This practice wastes the energy used in evaporating off the last portion of water and imposes a limit on the capacity of the evaporators. If the industry should decide to market the more stable 5-fold concentrate *(7)*, (so-called "superconcentrate") it would be impractical to go to 6-fold and use the juice cut-back process. Cold-pressed oils or some other concentrated flavor would be required for flavor fortification.

Much excellent work has gone into the study of the composition of orange, lemon, grapefruit, and lime peel oils *(2)*. Less has been done with the corresponding juices. Unfortunately, until recently the methods of separation employed in fractionating the oils have been both relatively crude and capable of damaging the more sensitive components. This has limited the scope and, to some extent, the value of the earlier work. Before Tswett's discovery of the chromatographic technique for separating materials on columns of powdered adsorbent, the flavor chemist in separating oil mixtures had to rely on vacuum fractional distillation and functional group reactions followed by fractional crystallization of the solid products.

Chromatographic separation on columns of adsorbent fortunately provides a very efficient and mild means of separation. This technique was adapted at the Pasadena Laboratory for the separation of the volatile oils in orange and grapefruit juice *(8)*, and these techniques since have been applied to the separation of components in cold-pressed lemon oil. A patent has been granted on the use of this method for the deterpenation of citrus oils *(3)*. In this deterpenation process a citrus oil is deposited on a column of powdered adsorbent and the terpene hydrocarbons are eluted from the column with a hydrocarbon solvent. The oxygenated terpenes which represent the concentrated flavor remain adsorbed

on the column. These latter compounds can be eluted from the column with more polar solvents such as ethyl acetate or ethyl alcohol and the mixture, after removal of the eluting solvent under vacuum, is the deterpenated oil. One company is known to be experimenting with this process.

Fractions from a chromatogram often contain more than one substance and so require further fractionation by rechromatographing, vacuum distillation, or separation by gas-liquid partition chromatography. For example, an apparently homogeneous aliphatic aldehyde fraction obtained from lemon oil by chromatography on a column of silicic acid was later separated into five fractions by gas-liquid partition. Infrared examination of these latter five fractions indicated that they still were not free of contaminating material (possibly hydrocarbons). Less difficulty was encountered in separating the volatile oils from grapefruit juice on silicic acid columns. The individual fractions from chromatograms of volatile oils from grapefruit juice contained only one compound as a rule, whereas, volatile orange oil fractions generally contained more than one compound, even though they appeared to be homogeneous on two dimensional silicic acid chromatograms (square sheets of glass coated with silicic acid). It was necessary, therefore, further to fractionate these mixtures by vacuum distillation on a micro column or by removing components as solid derivatives. These points are mentioned to emphasize that even with the elegant techniques now available the complexity of the mixtures in the citrus oils (at least orange and lemon) is such that no single method has proven to be adequate in itself for complete separation.

Detailed studies have been made at the Pasadena Laboratory using the columnar chromatographic techniques on the storage and processing flavor changes in hot-pack, single-strength grapefruit and orange juices *(4, 5, 6)*. In order to obtain enough of the volatile flavoring materials, it was necessary to process large quantities of juice (the juice contains about 0.1 percent oil). The first stage in flavor recovery; i.e., removal of water, was accomplished under reduced pressure (40-mm.) at about 45° C. (113° F.) in a circulating batch evaporator. In order to carry into the condensates the major portions of the volatile flavors, it was necessary to remove 50 percent of the water from the grapefruit juice and 75 percent of the water from the orange juice.

The condensates were divided into oil and water phases. The water phases were concentrated in a continuous vacuum stripping column and the resulting condensates were saturated with salt and extracted with ether. The combined ether phases were dried

over anhydrous sodium sulfate and carefully fractionated in a Podbielniak Hyper-Cal column. The volatile oils and water-soluble components were collected as condensate and further reduced in volume in a *continuous vacuum stripping column* packed with stainless steel turnings. The water phases of the final condensates and ice and dry-ice traps were saturated with salt and extracted with ether. The combined ether phases were carefully fractionated in a Podbielniak. The fractions from the Hyper-Cal column were analyzed, and from these fractions were obtained the data on the non-acidic, volatile water soluble components. The acids in the stripping column residues were neutralized with caustic and recovered as a mixture of solid sodium salts by evaporating off the water. The acids were then converted to the *p*-phenylphenacyl esters for chromatographic separation and identification.

The oily residues from the Hyper-Cal distillation were combined with the oil phases in the traps of the initial vacuum evaporation and flash-distillation steps; dried and separated by chromatography on columns of powdered silicic acid using hexane as developing solvent initially to remove the hydrocarbons as a mixture (i.e. to deterpenate) and subsequently using gradually increasing amounts of ethyl acetate in hexane followed by ethyl alcohol in ethyl acetate to complete the separation of the oxygenated fractions. The hydrocarbon composite was separated by vacuum distillation into a main fraction containing limonene, a lower boiling fraction, and a higher boiling fraction. The latter two fractions were rechromatographed on columns of silicic acid with hexane as developing solvent. The sesquiterpenes were separated from the higher boiling fraction and the terpenes other than limonene separated from the lower boiling fraction.

The results of analyses made on freshly reamed, freshly processed, and processed and stored single-strength grapefruit juice appear in tables 1, 3, 4, and 5. The results of analyses made on

Table 1. Volatile oil constituents of grapefruit juice

	Mg./kg. of juice		
	Fresh	Canned	Stored
Total oil	21.5	26.0	28.0
Hydrocarbons	17.5	19.7	12.5
Non-hydrocarbons	4.0	6.3	15.5

Table 2. Volatile oil constituents of orange juice

	Mg./kg. of juice		
	Fresh	Canned	Stored
Total oil	91.6	76.4	54.4
Hydrocarbons	88.4	71.0	46.0
Non-hydrocarbons	3.2	5.4	8.5

Table 3. Components of the volatile oils of grapefruit juice not altered by heating or storage

	Mg. oil/kg. of juice
Citral	0.06
Carvone	0.06
Linaloöl	0.12
$C_{15}H_{22}O$ (ketone)	0.55
Carveol	0.28
3-Hexen-1-ol	0.03
Dimethyl anthranilate	Trace
$C_{13}H_{15}N$	Trace
$C_{12}H_{20}O_2$ (ester)	0.20
Geraniol	0.02
Oxides	0.32

Table 4. Changes in concentration of water-soluble constituents of grapefruit juice

	Mg./kg. of juice		
	Fresh	Canned	Stored
Acetaldehyde	1.45	0.33	0.6
Acetone	None	None	0.1
Furfural	None	Trace	8.2
Ethanol	400	400	460
Methanol	0.2	0.2	23
Acetic acid	None	1.9	23.3
Acid A ($C_6H_8O_2$)	None	4.8	2.9
Acid B ($C_6H_8O_2$)	None	1.9	1.6

freshly reamed, freshly processed, and stored single-strength orange juice appear in tables 2, 6, 7, 8, 9, and 10. Approximately

Table 5. Volatile oil components in grapefruit juice altered by heating or storage

	Mg./kg. of juice		
	Fresh	Canned	Stored
D-Limonene	15.7	17.7	11.2
Beta-caryophyllene	1.4	1.4	0.88
Alpha-terpineol	0.03	0.88	2.02
Linaloöl monoxide	0.37	2.03	8.95

Table 6. Volatile water-soluble constituents in orange juice

	Mg./kg. of juice		
	Fresh	Canned	Stored
Acetaldehyde	3.0	3.0	0.8
Furfural	Trace	Trace	5.1
Ethanol	380	550	484
Methanol	0.8	Trace	62.0
Acetic acid	2.8	5.8	18.6
Propionic acid			0.1
Butyric acid			Trace
Iso-valeric acid			0.4
Acid A ($C_6H_8O_2$)	0.1	0.1	0.7

Table 7. Hydrocarbons in orange juice

	Mg./kg. of juice		
	Fresh	Canned	Stored
D-Limonene	80.1	63.3	43.0
Beta-myrcene	1.98	1.14	0.69
Alpha-thujene (?)	0.30	0.30	0.03
$C_{15}H_{24}$ (I)	5.80	5.78	2.04
$C_{15}H_{24}$ (II)	0.20	0.18	0.21

Table 8. Esters in orange juice

	Mg./kg. of juice		
	Fresh	Canned	Stored
Ethyl iso-valerate	0.01	0.01	0
Ethyl $C_6H_8O_2$	0.03	0.03	0
Methyl alpha-ethyl-n-caproate	0.06	0.10	0.02
Citronellyl acetate	0.10	0.04	0.02
Terpinyl acetate	0.08	0.01	0

Table 9. Carbonyls in orange juice

	Mg./kg. of juice		
	Fresh	Canned	Stored
n-Hexanal	0.04	0.03	0
n-Octanal	0.06	0.06	0
n-Decanal	0.05	0.04	0.02
n-2-Dodecenal (?)	0.06	0.06	0
Citronellal	0.04	0.04	0.02
$C_{15}H_{24}O$ (I)	0.14	0.10	0.15
$C_{15}H_{24}O$ (II)	0.07	0.12	0
$C_{15}H_{22}O$	0.09	0.09	0.09
Carvone	0	0	0.08

Table 10. Alcohols in orange juice

	Mg./kg. of juice		
	Fresh	Canned	Stored
Linaloöl	0.93	1.10	0.12
Alpha-terpineol	.32	1.72	4.08
n-Hexan-1-ol	.10	0.14	0.08
n-Octan-1-ol	.21	0.23	0.19
n-Decan-1-ol	.10	0.09	0.07
3-Hexen-1-ol	.10	0.18	0.06
$C_7H_{16}O_2$	.07	0.08	0.09
$C_{15}H_{26}O$ (I)	.07	0.24	0.23
$C_{15}H_{26}O$ (II)	.14	0.16	0
Polyoxygenated Cpds.	.12	0.15	0.75

2,500 gallons of juice was used in each set of determinations. The storage grapefruit juice sample was held at room temperature for 4 years, and the storage orange juice sample was held at room temperature for 3 years.

In general the changes in processing and storage for both juices appeared to result from acid-catalyzed hydrations. However, the specific changes in each juice were quite different. In grapefruit juice there was little change in total recoverable oil in the three samples (table 1), whereas in orange juice there was a 14 percent loss on processing and a 41 percent loss on storage (table 2). In both juices there were marked increases in furfural, methanol, and low molecular-weight fatty acids, particularly acetic acid (tables 4 and 6). The furfural is presumed to result from cyclization of sugars and the methanol to result from hydrolysis of pectins. Alpha-terpineol appeared in both stored juices (tables 5 and 10). An interesting and unexpected change is found in the processed and stored grapefruit juice samples. This is the almost quantitative conversion of the D-limonene lost

during storage to linaloöl monoxide (table 5). The fact that linaloöl monoxide is not greatly different in aroma from materials already present in the oils probably accounts for the acceptability of single-strength canned and stored grapefruit juice. No linalool monoxide was found in any of the orange juice samples. In stored orange juice, hydrocarbons, esters, carbonyls, and alcohols (with the exception of alpha-terpineol and some polyoxygenated compounds) all decreased (tables 7, 8, 9, and 10), some of them completely disappearing, so that there was a marked loss of the naturally occurring flavoring compounds. There was no linaloöl monoxide in the orange juice to mask off-flavors, so that products from changes in the water soluble materials; i.e., furfural and the lower molecular weight fatty acids, remained as the dominant flavor, or rather, off-flavor. The juice origin of the off-flavor was demonstrated by heating and storing juices after removal of the volatile oils.

Work on lemon oil is still in the preliminary stages. The study of lemon oil has been more extensive than the grapefruit and orange juice studies in that solid compounds also have been included in the program. The contribution of the solids to the characteristic ultraviolet absorption spectra of the oils and the possible contribution of these compounds to the dewaxing problem in processing oils warranted their investigation. The citrus oil study is easier in one respect in that the problem of removing enormous quantities of water in order to obtain material with which to work is eliminated. Preliminary fractionations of lemon oil have been made using the column chromatographic technique and gas-liquid partition chromatography. By this means between 20 and 25 fractions were obtained which appear to be homogeneous. As mentioned earlier, many of these probably contain two or more components. Several fractions deposited solids on concentration and cooling. To date 8 individual crystalline solid compounds have been isolated, 5 of which have been identified *(10)* (table 11). Three of the 8 solids are substituted coumarins,

Table 11. Substituted coumarins in lemon oil

	Approximate concentration mg./100 g.
5,7-Dimethoxycoumarin (Limettin)	53.0
5-Geranoxy-7-methoxycoumarin	116.0
5-Geranoxypsoralen	90.0
8-Geranoxypsoralen	59.0
Byakangelicin	9.0
Unknown coumarin I	3.0
Unknown coumarin II	10.0
Unknown furocoumarin I	2.5

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and the other 5 are substituted furocoumarins. Of the 5 compounds whose structures are known, 3 are geranyl ethers. The geranyl ethers are hydrolytically cleaved by weak acids yielding a phenol and geraniol, which in turn may be converted by acids to nerol (the *cis* form of geraniol). Some of the free geraniol and nerol reported to be in lemon oil actually may result from the cleavage of these coumarin ethers.

It is possible to determine the concentrations of the individual coumarins in lemon oil by chromatographing a small amount of the oil on glass strips coated with silicic acid, cutting and extracting the individual coumarin spots from the strip, and determining the quantity present by ultraviolet absorption measurement *(11)*. This technique offers a convenient method for determining the purity of an oil sample by reason of the fact that the distribution of coumarins is different for other citrus oils such as grapefruit, orange, and lime. The technique also provides a means for determining the degree of concentration obtained in a vacuum distilled "folded oil." In the "folded oils" the non-volatile solids are retained. In the latter application it is important to select one of the coumarins for comparison that is not altered or otherwise lost during vacuum distillation (some of the geranyl substituted coumarins are cleaved when heated *in vacuo*).

The typical aroma of lemon has been variously ascribed to citral and isocitral *(2)*. The greater sensitivity of isocitral to acid catalyzed hydration and eventual conversion to *p*-cymene by loss of water and the more pleasant fruity aroma of isocitral have suggested to some that the aroma of lemons is due to the presence of isocitral rather than citral. Lemon juice and lemon oil rapidly lose the characteristic lemon aroma when exposed to air and develop a so-called terpeny terebenthane type odor. This has been ascribed to the acid-catalyzed conversion of the citral or isocitral to *p*-cymene and to the oxidation of D-limonene to carvone and carveole.

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Discussion

DR. J. H. MITCHELL, JR. (QM Food and Container Institute):

I would like to know whether, as a result of some of the work which has been done on citrus juices, on chemical compounds which are present, and the chemical changes which occur in processing or storage, there have been objective methods worked out for the quality of these products or whether you feel that such methods will be worked out in the future?

STANLEY:

As far as I know, no good method has been developed. However, as you can see from the fact that most of the changes appear to be oxidative, methods which would serve for analyzing for aldehydes or for oxidation products may be promising. However, the industry is now going over to another type of juice, with the exception of the grapefruit juice, and so perhaps these methods would not apply to the concentrates.

MITCHELL:

Apparently the results along those lines would thus far be encouraging.

DR. J. S. BLAIR (American Can Company):

Don't you think that hydration and dehydration reactions would occur rather than oxidation?

STANLEY:

You brought out a point there—that many of these reactions, in connection with the terpenes, are actually hydration and dehydration reactions. I have not broken down the oxidation description to include that, but then I would consider hydration as a form of oxidation in this case.

QUESTION:

I would like to ask Dr. Stanley a question. It is true that Dr. Stanley said that a substance contains oxygen, whether oxygen has been introduced by actual oxidation or by addition of water, but, from a practical standpoint, we packers are concerned with what has happened to the product. It may be that the packer will be led down one trail in seeking remedial measures in connection with oxidation or he may be led down another trail if it happens to be something else and, therefore, I think that it is of some importance to the packer to know how these deteriorative reactions take place. I wonder if Dr. Stanley has any comment on that?

STANLEY:

I think that your point is a good one and that this is something that we should always keep in mind.

MRAK:

One of the gentlemen made a comment to the effect that heating improved flavor. People of the Western Regional Research Branch and others have shown that when you convert tomatoes into tomato juice people do not like the fresh taste, and that if the tomatoes are boiled the taste seems to be preferred. Is this due to change of flavor or due to reduction of the flavor substances which we get in a fresh tomato but not in such large quantities in the usual canned tomato juice?

STANLEY:

I have been told that in some of the processing plants they have tried to use a fast system for heating tomato juice and they have found that if they do this they end up with a juice that is not acceptable and one which has a vinegar type of flavor. Therefore, it looks as if you are losing something in connection with the improvement of the product.

MITCHELL:

Am I correct in assuming that tomato juice is usually preferred after a heating and processing treatment?

STANLEY:

I think that is generally accepted.

Preparation and Applications of Flavor Concentrates From Deciduous Fruits

RODERICK K. ESKEW

Eastern Regional Research Laboratory[a]

One factor which has contributed to advancing our knowledge of flavors, especially volatile fruit flavors, has been the development of effective means for recovering aromas in concentrated unaltered form. This has been the work of many individuals and groups. My own concern will be with the research carried out at the Department of Agriculture's Eastern Regional Research Laboratory in Philadelphia, where the first commercially practical process for aroma recovery was developed about a decade ago and where research in this field has continued to the present time.

Our work in the field of flavor recovery was undertaken to produce concentrated fruit juices without losing their aroma. This was finally accomplished by stripping the aroma from the juice under conditions to avoid flavor change, simultaneously fractionating the aroma to an essence, and restoring the essence to the vacuum concentrated juice. This is novel only in the means used to accomplish it. Since this is a flavor conference, my primary emphasis will be with regard to the essence recovery process,[b] its evolution during the past 10 years, as well as its likely potentialities for improving food flavors.

Early investigators concentrated the fresh fruit juice under vacuum and attempted to recover the volatile flavors by condensing them at low temperature; perhaps redistilling the condensate to further concentrate flavor. Although logical from the viewpoint of avoiding flavor damage, the use of vacuum has one important drawback. All fruit juices contain dissolved air or other gases, and in any flavor recovery system they must be vented since they do not condense. Unless special precautions are taken, such as I will discuss later, this gas leaves the system carrying some aroma with it. The loss of volatiles in the vent gas is roughly in inverse proportion to the absolute pressure at which the system is operated. For example, when the gases are vented from a system under a 27-inch vacuum (3-in. absolute pressure) the losses are more than 10 times as great as when venting at atmospheric pressure. This is true even when the gases are in both cases

[a] A laboratory of the Eastern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture.

[b] The term "essence" as used here refers to a distilled aqueous solution of aromas more concentrated than in the parent fruit.

chilled and inleakage of air is neglected. In practice there would be some inleakage of air which would further exaggerate this difference. This factor of vent gas loss can be serious when the amount of vent gas is high as a consequence, for example, of a slight fermentation of the juice. Vent gas losses also are magnified with increasing concentration of the distillate and, of course, with increase in volatility of the product. It is always the top notes which are first lost in such a system, throwing the flavor out of balance.

Early Design

With these objections to vacuum in mind, Milleville and coworkers at the Eastern Laboratory in 1944 designed a unit to operate at atmospheric pressure and above.[c] I think I can safely say that with this apparatus they were the first to recover apple essence in concentrated, substantially unaltered form. This enabled us to make a full-flavor apple juice concentrate which gave a delightfully fresh flavor on reconstitution. Figure 1 shows the arrangement of this apparatus. I want to devote a few minutes to it because it was the progenitor of many flavor recovery units of improved design. The juice, in this case apple juice, was fed at a constant rate to a super-heater wherein its temperature was raised in about 3 seconds to 320° F. Flashing the heated juice to atmospheric pressure was sufficient to vaporize about 10 percent. Previous investigators had shown that all the volatiles in apple juice appeared in the first 10 percent evaporated. The liquid and vapor were separated in a vapor-liquid separator, the stripped juice passing to a vacuum evaporator for rapid cooling and concentration. The vapors, now concentrated about 10-fold, passed to a fractionating column for further concentration to the desired degree. Usually this was 100-fold, that is, the aroma was collected in a volume corresponding to 1/100 that of the starting juice. The vapors issuing from the top of the column were condensed, a part being returned as reflux and the remainder being drawn off at a metered rate with respect to the juice feed rate. The product was apple essence which, when restored to the concentrated stripped juice, gave the full-flavor product mentioned above.

Our success with apples suggested that volatile flavor recovery might have much broader applications. However, although the

[c] "Recovery and Utilization of Natural Apple Flavors," by Howard P. Milleville and Roderick K. Eskew, AIC–63, September 1944. Also in the Fruit Products Journal and American Food Manufacturer, vol. 26, pp. 48–51, October 1944.

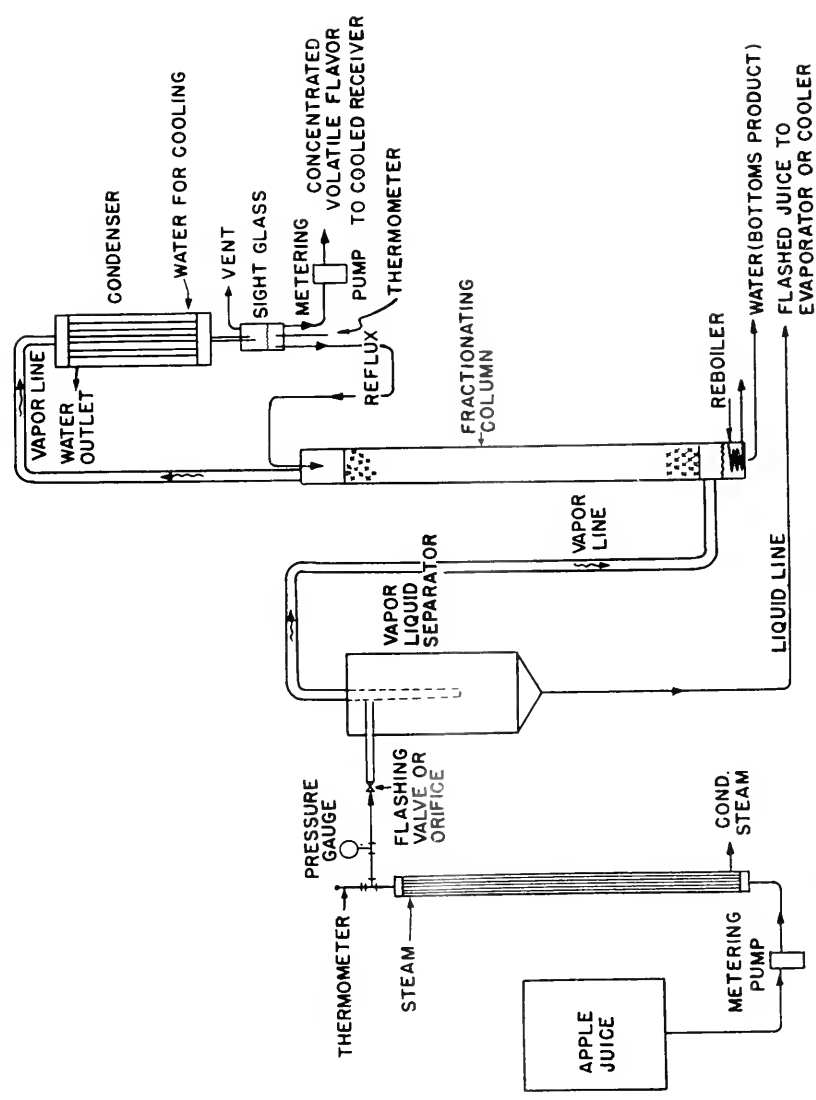

Figure 1. Diagram of process for recovering volatile apple flavor.

basic principles of the process were found to be applicable to the juice of many other fruits, the early apparatus designs gave mediocre results in most cases. Even when a rapid evaporator was substituted for a super-heater and when the vent gases leaving the system were thoroughly chilled with the volatile fruit concentrate before being vented, the apparatus was still unsatisfactory for some fruit juices. In some cases the juice flavor was altered by the heat incident to vaporizing the volatiles, and in others the desirable top notes were lost.

Improved Design Extends Utility

The arrangement shown in figure 2 is much more versatile than the earlier designs and can be used with all but the most heat-sensitive juices. The juice is fed by a positive delivery pump at a constant rate to an atmospheric preheater where its temperature is brought approximately to its boiling point. It then passes into the vaporizer where the amount required for aroma release is vaporized. This varies widely depending upon the fruit. For apples it is 8 or 10 percent and, for grape juice, 40 or more percent. This separation of the heating and vaporization steps permits accomplishing the aroma release with the minimum heat effect. The preheater consists of a small-diameter tube through which the juice travels at such velocity that it is in turbulent flow and, in consequence, is rapidly and uniformly heated. If the velocity in this tube is not less than 20 feet a second, fouling of the tube walls, which resulted with apple juice in earlier designs, is eliminated. In this arrangement the juice can be heated, the desired percentage vaporized, and the juice again cooled by flashing into vacuum in a total time of about 3 seconds. This is very much faster, and hence there is less heat damage than in earlier designs.

The aroma-bearing vapors enter a fractionating column where they are concentrated. They then are condensed and a portion is drawn off as product. You will note, however, that there is quite a different method of handling the non-condensable gases before they are vented. Instead of releasing them directly to the atmosphere or chilling with essence as was done in earlier designs, they are countercurrently scrubbed with cold water. In this case the scrubbing liquid is column bottoms. This means they leave the system in equilibrium with water and hence are free of aroma. In earlier designs when the gases were cooled with essence they were vented in equilibrium with aroma-rich liquid.

The scrubbing liquid, containing whatever top notes it has picked up from the vent gases, is returned to the column. Because of this liquid feed, we require a stripping section in the column where formerly it was not necessary. We have used this arrangement with good success in making essences from apples, grapes, cherries, blackberries, blueberries, strawberries, and other fruits. It should be applicable to many other juices and will permit the making of high-fold essences with good recovery because of the system used in treating the vent gas.

Vacuum Operation Required for Some Juices

There are juices which are too heat sensitive to be handled at atmospheric pressure, notably orange juice. The whole system then must be operated under vacuum. This is not merely a matter of attaching a source of vacuum to a unit designed for atmospheric use. Although the basic steps in the process of flavor recovery remain the same, the apparatus must be modified chiefly as regards the relative size of its component parts. For instance, if the system is to operate at the same juice rate and under 3 inches absolute pressure, the fractionating column and some other vapor handling parts must be enlarged to accommodate about 10 times the volume of vapor that they were required to handle at atmospheric pressure.

With vacuum operation we must, of course, be especially careful to avoid aroma loss in the vent gas. This can be prevented by scrubbing the gas with chilled column bottoms as shown in figure

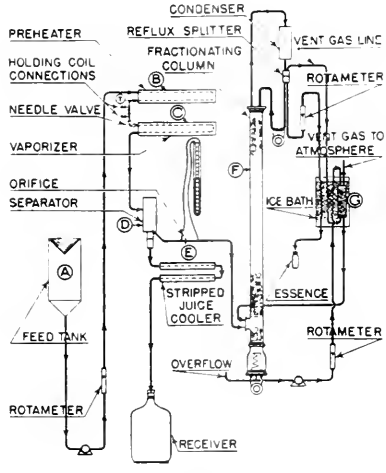

Figure 2. Improved apparatus for reducing vent gas losses.

2 although, of course, the scrubbing system must be enlarged to accommodate the increased volume of gas at the lower pressure. We built such a unit on a pilot-plant scale. It was used at our Bureau's Winter Haven, Florida, laboratory and could strip and concentrate the aroma of orange juice without heat damage to the juice. However, there is some question as to the practical value of the essence so recovered.

Single Pass Atmospheric Concentration

It was stated earlier that 40 or more percent vaporization is necessary to release aroma from Concord grape juice. Methyl-anthranilate, the component that characterizes Concord grapes, is tenaciously retained in the juice. A residual aroma still can be detected after 60 or more percent of the liquid has been vaporized. This adversity can be turned to advantage by combining aroma release and juice concentration into a single operation.

Naturally, if a high density concentrate, for example 7-fold, is to be made, the juice first must be depectinized. This can be done conveniently simultaneously with detartration.

Figure 3 illustrates how such an apparatus is used. The detartrated, depectinized juice is preheated and passes to the vapor-

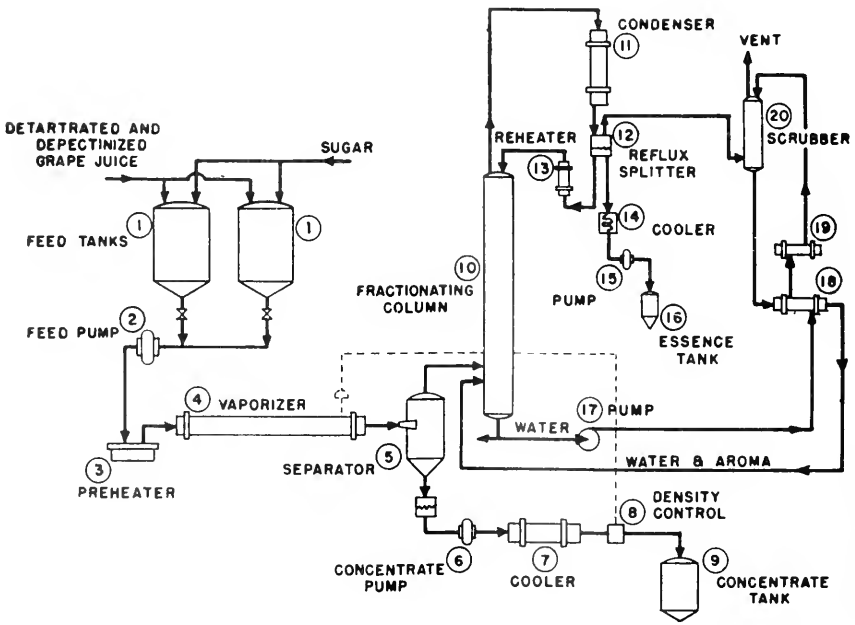

Figure 3. Single pass atmospheric concentration of grape juice.

izer in the conventional manner. At this point, however, instead of vaporizing 40 or 50 percent all the liquid is driven off necessary to concentrate to about 75° Brix. The essence recovery apparatus is substantially the same as that we have already described but somewhat larger in proportion to the vaporizer because of the greatly increased amount of vapor to be handled. Any methylanthanilate remaining in the concentrate will appear in the finished product, and if the recovered essence is restored the product will be approximately full flavor. I qualified that term "full-flavor" deliberately, for our tests have shown that less than half of the methylanthranilate which goes to the fractionating column actually appears in the essence. The remainder is lost in column bottoms. Fortunately a little bit goes a long way, so that this much loss is not serious from a practical standpoint. We recognize, however, that there is a need to improve the system for recovering Concord grape essence and are now studying the problem.

Heat Effect Sometimes Desirable

In contrast to juices which must be handled under vacuum, there are others in which the desired flavor can be intensified by deliberate heating. Montmorency and Morello cherry juices are in this category. We have found that if Montmorency cherry juice is heated for 1¼ minutes at 230° F. it will develop more aroma. If the aroma is then stripped and restored to the juice concentrate, the product will have much more cherry flavor than if made from unheated juice. This isn't very surprising for we all know good cherry pie has more of what we call cherry flavor than does the raw fruit itself.

Concord grape juice also benefits flavor-wise from some degree of heat. Flavor recovery studies made at various stages of conventional manufacture show a progressive flavor enhancement up to and through the aging process for tartrate settling. Undoubtedly some other fruits would be better for heat treatment. We are all familiar with flavor development by the roasting of coffee beans, the fermentation of vanilla pods, the aging of whisky, and the cooking of many foods. I wonder if we have yet exhausted the possibilities of flavor enhancement (and I mean either modification or intensification) in the case of fruits. I am told that in Europe volatile fruit flavors may be altered deliberately during distillation by a catalysis which favors ester formation. Thus, the latent aroma of unripened fruit may be said to develop during

recovery to a semblance at least of that found in the sun-ripened product.

I have talked a great deal about fruit juices, for most of our experience has been in this field. To an ever increasing extent, juices are being sold in the form of concentrates. If these concentrates are made by vacuum evaporation they will not yield juices of satisfactory quality unless the volatiles are restored. In the case of citrus juices, this is of course done by adding some fresh cutback juice to the concentrate. This has proven satisfactory because the chief flavoring constituent here is in the peel oil contained in the cutback juice. However, if fresh flavor is to be had in high-density (e.g. 7-fold) concentrates made from non-citrus juices, essence must be restored. If then the volatiles must be restored to the juice concentrates, how will they ever appear on the market as essences for use by manufacturers of extracts, beverages, candy, or other food products?

Volatile Fruit Concentrates from Preserve Manufacture. The answer may lie in the recovery of essences from vapors given off from vacuum preserve kettles. It is necessary only to replace the jet condensers with surface condensers and to install storage tanks for the condensates. These would then be processed in essence recovery equipment somewhat simpler in design than the arrangement we saw in figure 2. This process is now in commercial use. The aromas can be returned to the preserves to enhance flavor. This practice was referred to recently in the advertisements of a large preserve manufacturer. Sometimes the essences are sold to flavor other food products; for example, milk drinks, ice cream, or candy. The condensates accumulated from a few days' operation in a preserve plant can provide enough essence to permit extensive composition studies. Advantage has been taken of this fact by several groups studying the composition of strawberry flavor. Naturally, preserve essences partake of the character of both the fresh and cooked fruit.

There is another source for apple essence at least, and that is the juice of peels and cores of sound apples used in making vinegar. Volatile concentrate can be recovered profitably from such juice without impairing the quality of the vinegar, affording the manufacturer another source of income.

Powdered Fruit Juices. If 4-fold fruit concentrates are good and 7-fold fruit concentrates better, why not remove all the water and produce powdered juices? This has been done commercially with citrus juices. More recently a comparatively simple method has been developed on a pilot plant scale at the Philadelphia labo-

ratory. It is applicable to the less heat-sensitive juices such as apple, grape, cherry, and probably others.

Three major problems must be solved if the powder is to yield a good juice on reconstitution. First, the water must be removed under conditions which will not damage the flavor; second, the aroma must be recovered and restored without re-introducing an objectionable amount of water; and third, the product must be noncaking and stable on storage.

The first steps in the process are the same as those for making a full-flavored concentrated juice. That is, the aromas are stripped off and concentrated to an essence of about 150-fold, and the stripped juice is then vacuum concentrated to about 75° Brix. By 150-fold we simply mean that the aroma in the juice has been concentrated to a volume 1/150 of that of the juice. This is about the strength of the fruit essences of commerce. Obviously, it is much too dilute for restoration to a powdered juice as too much water would be introduced. It is necessary to fractionate 150-fold essence to approximately 1,000-fold. This is usually done in an efficient laboratory column as the volumes involved are relatively small.

Using the 75° Brix juice concentrate and 1,000-fold essence, powdered juices are made as shown in figure 4. The concentrate is fed through a metering pump to a preheater where its tempera-

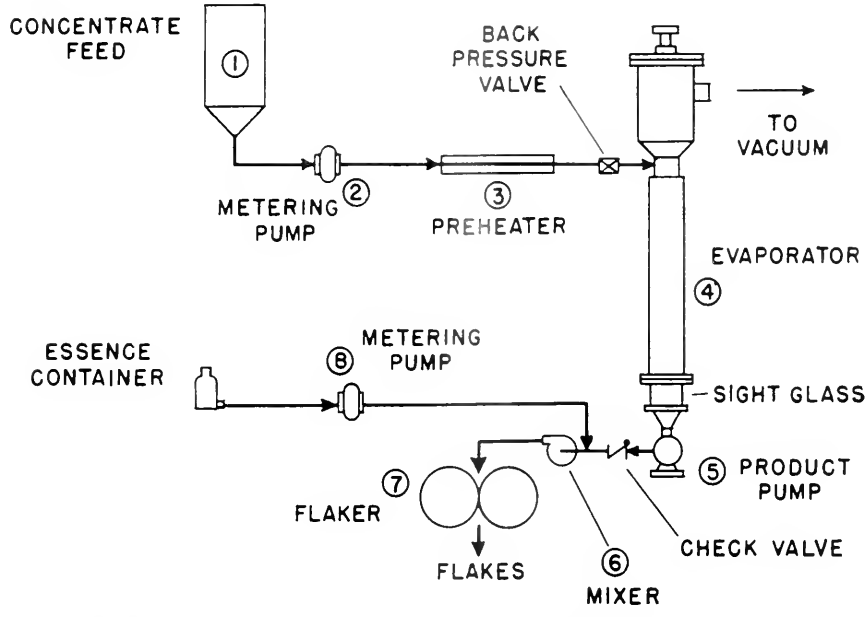

Figure 4. Apparatus for continuous dehydration of fruit juices.

ture is raised to about 135° F. using vacuum steam as the heating medium. Passing through a check valve it enters a vacuum evaporator of special design. The unit has a rotor operating at such a speed that the viscous concentrate is kept in a thin film on the heated walls. With steam of 10 pounds per square inch in the jacket and a vacuum of about 27 inches of mercury, concentration may be carried in a few seconds to approximately 98 percent solids. Under the vacuum mentioned, the product will be at about 230° F. as it leaves the evaporator and, in effect, is a molten powder. It is pumped out by a positive delivery pump. The 1,000-fold essence is now introduced at a metered rate on the positive delivery side of the product pump. Although the temperature of the concentrate is far above the atmospheric boiling point of the essence, the essence does not boil at the point of its introduction as the system is under positive pressure. Immediately following the point of introduction, a mixing device such as a centrifugal pump intimately blends the essence and concentrate and discharges it to flaking rolls. Obviously the distance between the point of discharge from the evaporator and the chilled rolls must be kept at a minimum to avoid heat damage. The flaked product can be easily crushed to a readily soluble powder which on dissolving in cold water yields a fruit juice possessing to a surprising degree the flavor attributes of the fresh fruit juice.

A very small amount of water is inevitably introduced with the essence, but this only raises the moisture content from about 2 percent to 2½ percent. The powders, of course, are very hygroscopic and must be handled in a low humidity atmosphere. If they are packaged with a desiccant, they can be stored for more than a year with practically no change in flavor. Even at 100° F., the flavor stability is excellent and caking will not occur after the desiccant has reduced the moisture to about 1½ percent. This may require several weeks storage at room temperature.

In conclusion I think it can be said that this development of commercially practical methods for aroma recovery has made available concentrated volatile flavors in quantity to permit extensive study of their composition, and it has enabled the production of ice cream, milk drinks, candy, jellies, and other food products possessing fresh fruit flavor. It also may have pointed a way toward concentrated and powdered coffee and tea of better flavor.

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Discussion

QUESTION:

I would like to ask Mr. Eskew if he has had any experience in connection with the Muscatine grapes from the southern part of the country.

ESKEW:

No, our only experience with grapes has been with the Concord grape.

MITCHELL:

I might ask Mr. Eskew about his statement concerning the deliberate attempt to alter flavor of juices by increasing the ester formation. I wonder if you would go into that a little bit more?

ESKEW:

I wish that I could go into it a little more. Unfortunately, the only information that I have on that is very fragmentary. I recently had a talk with this gentleman from Holland, but he did not mention anything that I could add to what I have already said.

The Volatile Flavors of Strawberry[a]

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Western Utilization Research Branch
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Interest in the volatile flavor constituents of strawberries reflects the need for objective methods of assaying flavors. In the large scale fruit-breeding programs underway, in the determination of maturity, and especially in processing and in the post-processing history of fruits, we must know what causes the char-

[a] Presented at the Symposium on Chemistry of Natural Food Flavors by Dr. Corse.

acteristic flavor and the chemical changes that occur which affect flavor. The volatile strawberry-flavor problem, and indeed most fruit-flavor problems, are difficult in that the flavor is an exceedingly complex mixture of chemicals in very dilute solution. Further, these organic chemical compounds responsible for the strawberry fresh-fruit flavor vary greatly in their amounts, their volatilities, and their flavor intensities.

In order to start on the problem, it is necessary to separate the responsible chemicals from the great amount of contaminating water, fruit solids, and the bulk of volatile insipid organic material. Two methods have been used in strawberry-flavor research: extraction and distillation. The classic work on volatile strawberry constituents was done by Coppens and Hoejenbos about 1930 *(1)*. They extracted 445 kg. of strawberry juice (Scarlet variety) with ether in a continuous extractor, distilled off most of the ether, removed acidic materials with sodium carbonate solution, and dried the remaining ether solution. After concentration there was left 86 g. of a neutral oil grossly contaminated with non-volatile waxes. They finally obtained a volatile fraction which was distilled at various pressures. Unfortunately, no mention was made of the type of distillation apparatus used, but considering the time and place, it must have been of very low plate efficiency. Nevertheless, by repeated careful distillations, the use of qualitative tests, and the preparation of derivatives, Coppens and Hoejenbos were able to identify a number of components and to have reasonable approximations or educated guesses as to others (table 1). A mixture of these materials, however, certainly does not have the characteristic odor of strawberries.

Table 1. Volatile flavor components from "scarlet" strawberry (1)

Acetic Acid	Ethyl or Methyl Caproate
n-Caproic Acid	Ester of Butyric Acid
Cinnamic Acid	n-Hexanol
Ethanol	dl-α-Terpineol
Ethyl Acetate	l-Borneol
Isoamyl Alcohol	Terpin

In order to avoid the initial extraction of non-volatiles which caused Coppens and Hoejenbos considerable trouble, work at the Western Utilization Research Branch has been based on distillation techniques followed by extraction. About half the weight of berries was collected as water solution from strawberry puree in a vacuum flash evaporator or from whole berries in vacuum-pan jam manufacture. These aqueous solutions were further concen-

trated in a stripping operation to about 60-fold.[b] This sort of low plate-efficiency process cannot be carried very far, because the high-boiling materials form azeotropes which boil very close to 100° C. and pass off with the water. For further concentration, a high-plate efficiency, continuous feed distillation column was designed and used *(2, 3, 4)*. It thus was possible to obtain concentrates above 600-fold wherein there was no observable damage to flavor. This concentrate (A) was used for analysis of various components (fig. 1). Most of the material, however, was subjected to further distillation. The low-boiling (to 85° C.) fraction (B) was collected and the rest distilled as azeotropes. The high-boiling azeotropic solution then was extracted with isopentane in a continuous extractor to yield, after working up, a water-insoluble oil (C) bearing the characteristic fresh-strawberry flavor. The amounts of this oil varied from 1 to 7.5 p.p.m. of original strawberry. Under these conditions of preparation, it contained about one-third free fatty acids and actually represented less than 10 percent of the volatile carbonaceous substances. Investigations of concentrates (A) and (B) above have led to the identifications given in table 2.

Table 2. Analysis of fresh Marshall strawberries

Description	P.P.M. in original puree
Essence (as carbon)	42.0
Acetaldehyde	4.9
2-Hexenal	7.2
Acetone	2.7
Biacetyl	0.21
Ethanol	45.5
Methanol	4.7
Esters (calc. as ethyl acetate)	9.4
Water insoluble oil	7.5
Free fatty acids	3.00
n-Caproic	1.5
n-Valeric	0.8
n-Butyric-Isobutyric	0.52
Acetic	0.12

Except for the free fatty acids in the oil (C), this fraction was almost intractable. Attempts to distill it under reduced pressure in a low-holdup spinning band column were abortive. Visible deterioration occurred in reaching equilibrium, which required at least 2 hours, and the distillation was discontinued because of extensive decomposition after 7 or 8 hours. The odor of the dis-

[b] The word "fold" as used here means essentially the extent of concentration. For example, it would take 2,000 g. of puree to make 1 g. of a 2,000-fold concentrate.

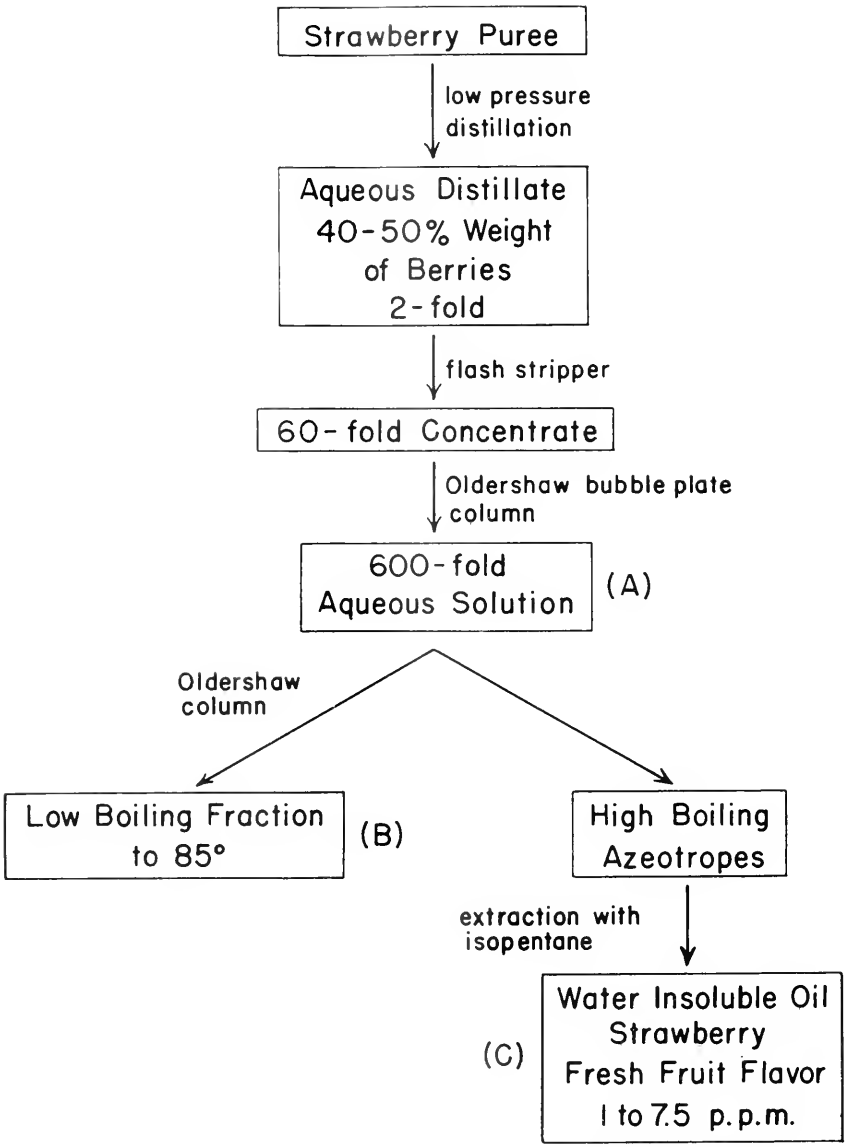

Figure 1. Concentration procedure for low boiling fraction (B) and the oil flavor fraction (c).

tillate, although pleasant, was not especially reminiscent of strawberries, and the residue was acrid.

The development of gas-liquid partition chromatography (GLPC) by James and Martin *(6)*, and the successful use of thermal conductivity *(7)* for detecting the fractions as they leave the apparatus led us to adopt this powerful tool in the investigation of the essential oil from strawberries *(7)*. In GLPC a col-

umn is packed with a stationary phase of size-graded solid particles wet with a liquid of low vapor pressure. The components of a mixture placed on the column are eluted by a means of a gas, and separation occurs as a result of differences in vapor pressures over the liquid phase. The thermal conductivity cell detects contaminants in the effluent gas, and their presence may be plotted on a recording potentiometer as they emerge from the column (fig. 2).

Gas chromatography represents a superlative method of separating small amounts of volatile materials. Separations may be

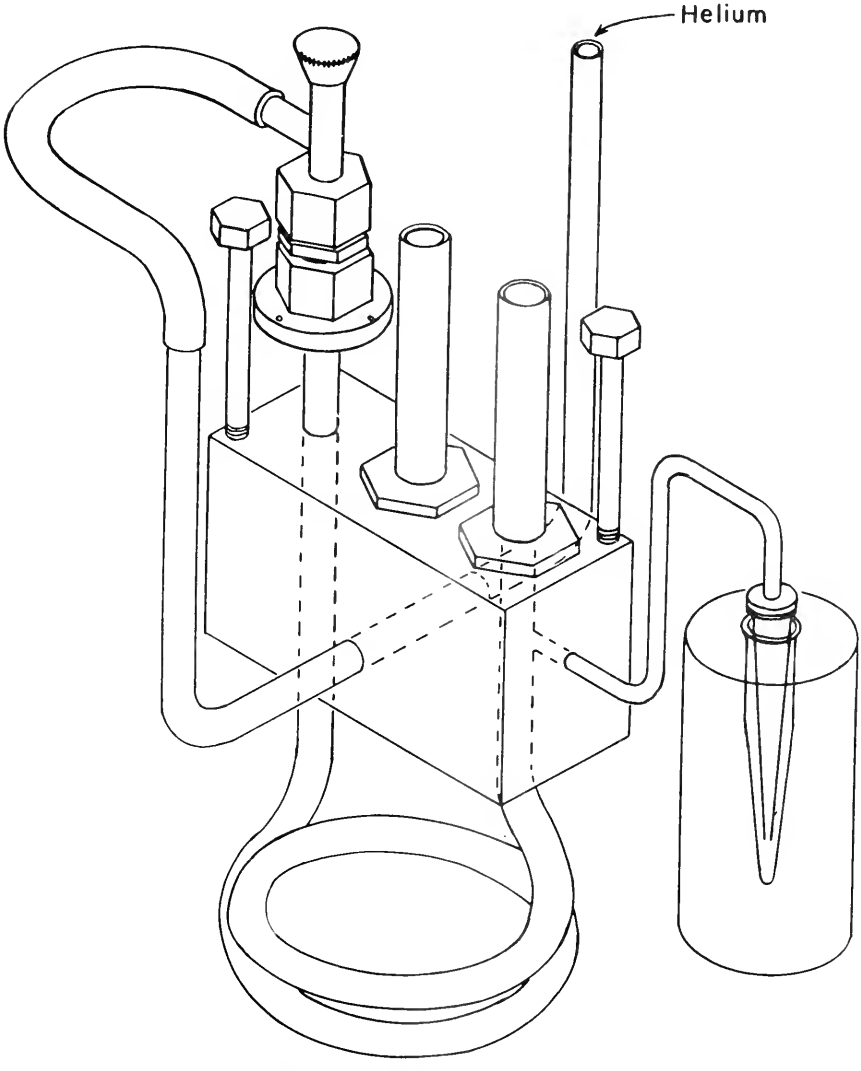

Figure 2. Isometric drawing of assembled GLPC Unit.

accomplished in 10 minutes on a 4 mg. sample and, by means of a special collecting device, near quantitative recoveries were made of materials boiling as low as 27° C. (80.6° F.). The pen recorder provides a permanent record of the emergence of each substance in the form of a peak whose area is proportional to the molar quantity of the substance. In a homologous series, the logarithm of the time for a peak to appear (retention time) is proportional to the boiling point. Thus, a close estimate of the boiling point of an unknown may be made from the calibration curves of known materials.

The resolving power of this apparatus is very high. Using an 11-foot column, ¼ inch in diameter, packed with a Celite-silicone-stearic acid mixture, James and Martin estimated the efficiency of the column at approximately 2,000 theoretical plates. In addition to its speed and phenomenal plate efficiency, this method of separating volatile substances has a great advantage over distillation in that the type of stationary phase may be changed by packing a new column. This, in effect, gives a situation analogous to 2-dimensional paper chromatography. The two liquids, water and methanol, appear as a single substance when subjected to separation on a silicone column, but when placed on a Carbowax column, this mixture was separated cleanly.

A further point should be emphasized: materials of different types, such as alcohol and esters, may be separated readily, although their boiling points are almost identical. For example, ethanol and ethyl acetate are separated on either the silicone or the Carbowax column, while their boiling points differ by approximately 1°. Another distinct advantage of the method is the absence of any tendency to form azeotropes.

A sample of strawberry oil, injected into a 10-foot silicone column operating at a temperature of 200° C., with a helium flow rate of 42 ml./min., gave a serrated curve (fig. 3) indicating an extremely complicated mixture. Eight arbitrary fractions were collected, and the lower boiling ones were re-run at a lower temperature and further collections again made (fig. 4).

None of these silicone fractions appeared to be homogeneous. When S–1, the first silicone fraction, was re-run on a Carbowax column, a striking result was obtained, as shown in figure 5. Peak 13, the largest, was identified chemically and physically as isoamyl alcohol. The quantities of most of the other substances precludes their identification by chemical means, and mass spectra are being run for this purpose.

The second silicone peak, S–2, likewise was shown to be a complex mixture when run on a Carbowax column (fig. 6). The two

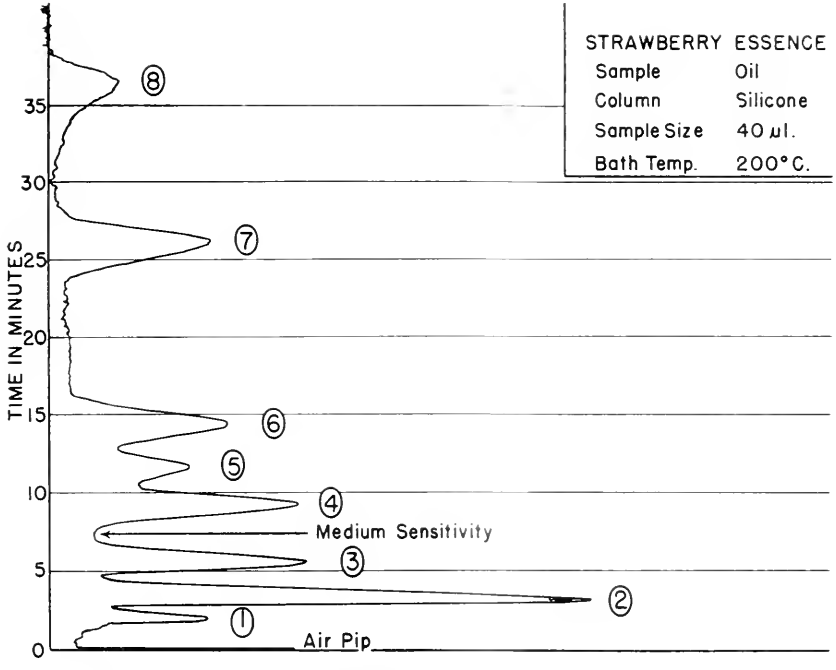

Figure 3. Eight major fractions S-1 to S-8 separated from strawberry oil by GLPC.

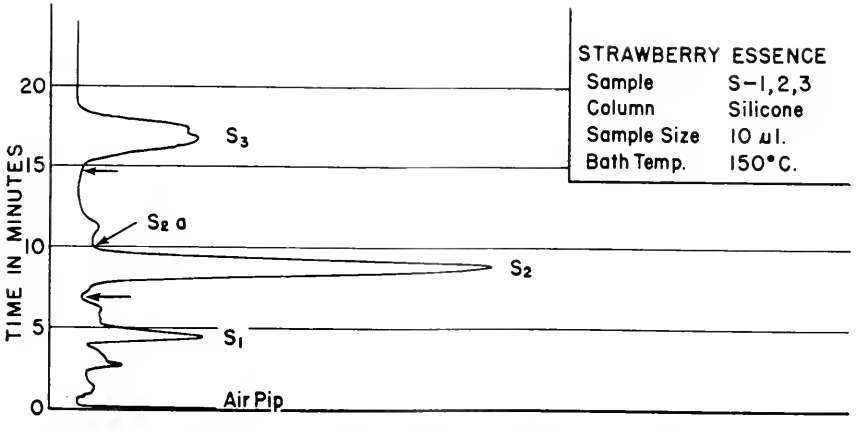

Figure 4. Fractions S-1, S-2, and S-3 re-chromatographed at 150° C.

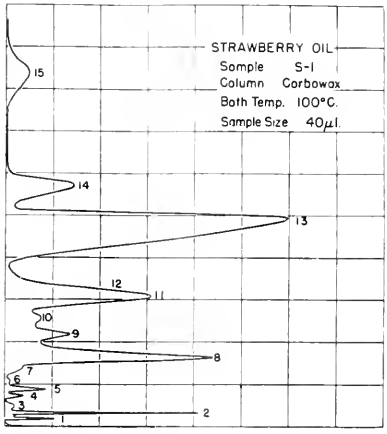

Figure 5. Fraction S–1 chromatographed on a carbowax column.

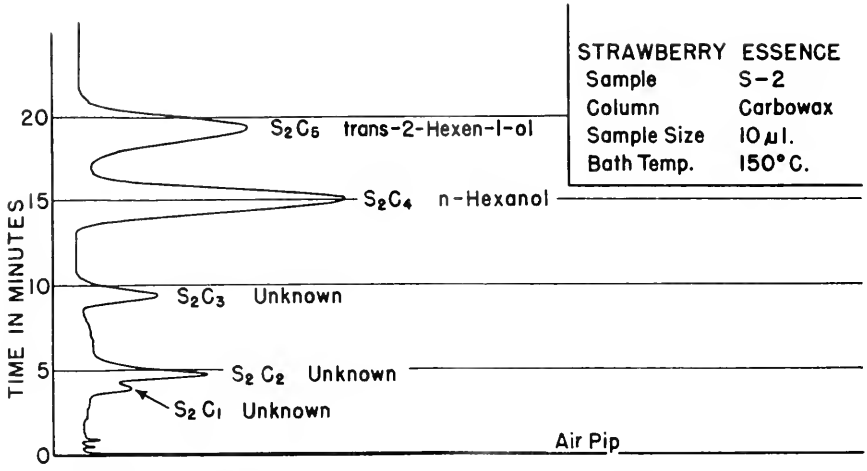

Figure 6. Fraction S–2 chromatographed on a carbowax column.

major components are *n*-hexanol (S–2, C–7), previously found by Coppens and Hoejenbos, and *trans*-2-hexene-1-ol (S–2, C–8). This latter alcohol has not been reported previously as a natural product, although both *cis* and trans-3-hexene-1-ols do occur in nature. *Trans*-2-hexene-1-ol has a remarkably pleasant odor, reminiscent of green apples. The occurrence of *trans*-2-hexene-1-ol and *trans*-2-hexenal, are, of course, related, but in an unusual manner. If berries are frozen or heated prior to being pureed, no *trans*-2-hexenal is found. However, fresh crushed berries do

yield large quantities. We believe *trans*-2-hexenal to be an artifact. Its formation can be explained if heat or freezing disrupts the mitochondria in such a manner that the normal dehydrogenase activity necessary to form the aldehyde from the alcohol is destroyed.

The third silicone peak, S–3, is resolved into many compounds on Carbowax columns (fig. 7). So far ethyl caproate (S–3, C–4),

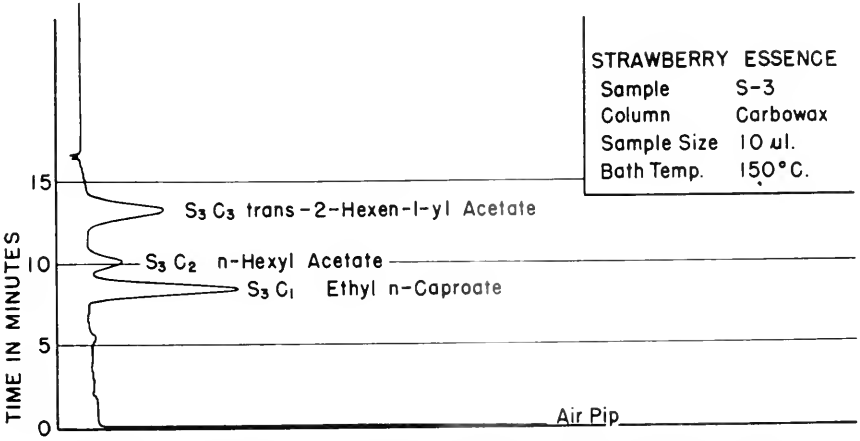

Figure 7. Fraction S–3 chromatographed on a carbowax column.

n-hexyl acetate (S–3, C–5), and *trans*-2-hexene-1-yl acetate (S–3, C–6) have been identified. The hexenyl acetate, probably the most pleasantly fruity component in the oil, has not been reported as naturally occurring.

The last peak on the original silicone columns, S–8, contains about half ethyl cinnamate (table 3). This is a small peak and, like the others, a mixture. Much work remains to be done to

Table 3. Partial composition of water-insoluble oil (strawberry fresh-fruit flavor)

Isoamyl Alcohol	n-Hexyl Acetate
trans-2-Hexene-1-ol	Ethyl Caproate
n-Hexanol	Ethyl Cinnamate
trans-2-Hexen-1-yl Acetate	(trans-2-Hexenal)

identify the many unknown components which in total represent less than 20 percent of the strawberry fresh fruit flavor. Although any one of these is quite minor in relation to the whole, the presence of a number of them is necessary for the characteristic aroma.

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IV. FLAVORS OF PROCESSED FOODS

Cheese Flavor

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Cheesemaking over the ages has been classified as an art. Only recently has an association with science become apparent. The evolution has been slow, but progress has been steady, and now there is no hesitation in stating that a cheesemaker is employed both in the arts and sciences.

The development of a ripened cheese begins, initially, with a rapid and progressive dehydration of the fresh curds from coagulated milk and a working-up of these curds into a homogenous compact mass. Control of physical properties of the curds in the cheese vat through heat, acid, and salt manipulation are vital to the process. The shape of the cheese is molded by hydraulic presses, and the body and flavor are developed over a period of time—the ripening period—in specially designed rooms held at specific temperatures and relative humidities.

Milk from most mammals can be used for cheese. In practice, the milk of the cow, goat, sheep, buffalo, mare, and camel are utilized in various areas of the world. The flavor of the resulting cheese is influenced to some extent by the milk source.

Characteristics of Cheese

Cheese, with Cheddar as a classic example, presents a fascinating panorama of fermentation patterns in which a multitude of chemical reactions are proceeding with great intensity. To initiate these reactions are tremendous concentrations of diverse, living bacteria, perhaps more than can be found in any other natural food. Numbers as high as 10 billion bacteria per gram have been observed in green cheese.

The interior of most ripened cheese is acid, pH 5.0–5.6, moderately salted and saturated with carbon dioxide with little or no oxygen. Such cheese as Camembert and Brie, however, may have some ammonia development because their higher pH allows considerable deaminase activity. In the cheese environment, considerable energy sources exist for bacterial activity, but not all bacteria grow well during the ripening period because of the ob-

vious deterents of an anaerobic condition, the effect of salt and acid, and the presence of other inhibitory substances.

Increased Knowledge of Cheese Components

One past stumbling block to productive research on cheese flavors was due to the meager information available concerning chemical products of cheese ripening. The application of chromatographic techniques since 1949 by American and European investigators has greatly improved this condition. Now fairly well established are the identity and rate of formation of 18 to 20 free amino acids and amines of Cheddar cheese and the type and quantity of free fatty acids (tables 1 and 2). It is interesting

Table 1.[1] *Free amino acid and amine composition of raw- and pasteurized-milk Cheddar cheeses*[2]

Amino acids	Raw-milk cheese 60 days at 60° F.	Pasteurized-milk cheese 60 days at 60° F.
	(Mg./g. cheese)	
Glutamic	4.93	1.03
Aspartic	0.56	0.17
Leucine-Methionine	2.46	0.95
Basic	5.38	5.83
Valine	3.37	3.29
Alanine	0.64	0.62
Glutamine	0.42	0.58
Serine	0.03	0.27
Phenylalanine	2.22	3.23
Tyrosine	0.18	0.97
Glycine	0.08	0.08
Threonine	0.54	0.19
Proline	0.11	0.00
α-amino-butyric	0.05	0.00
Cysteic acid	0.00	0.00
γ-amino-butyric	+++	++
Methionine Sulphoxide		
Asparagine	0.00	> 6.06

[1] From *J. Dairy Sci.*, *34*, 235 (1951).
[2] Both cheeses made from same herd milk.

Table 2. The free fatty acid concentration of Cheddar cheese at the end of 200 days ripening—40°–49° F.[1]

Fatty acid	Raw-milk cheese	Pasteurized-milk cheese
	μ moles acid per 100 g. cheese	
Acetic	350	350
Butyric	450	325
Caproic	50	50
Caprylic	225	100
Capric	100	25

[1] Interpolations from graph. *J. Dairy Sci.*, *32*, 864 (1949).

to note that the free amino acids appear early in cheese, some as leucine, glutamic acid, and valine, within 48 hours after manufacture. Also, a few significant chemical differences between the composition of pasteurized milk cheese and raw milk Cheddar cheese have been unraveled with the new analytical techniques. Raw milk cheese exhibits more total free amino acids and fatty acids than pasteurized milk cheese at comparable periods of ripening, but the latter, generally, is significantly higher in serine, asparagine, and tyrosine *(11)*. Even so, not all of the full composition of any of our natural cheese is known, though Cheddar cheese is most completely defined, followed by native Swiss.

In a ripening cheese are found the major milk constituents—fat, protein, lactose—and many products of their hydrolysis. Yet, even as the latter are formed, strong active forces are engaged in converting these to new compounds. The amino acids, for example, are transformed in part through decarboxylation and deamination to amines, amides, and fatty acids, *i.e.*, aspartic acid to asparagine, tyrosine to tyramine or phenol, glutamic acid to γ-amino-butyric acid, and lysine to cadaverine. There are other examples. Also, durable micro-organisms, surviving in cheese for weeks, may utilize certain of the free fatty acids and amino acids, thus removing from circulation compounds created during the earlier stages of ripening. Throughout the entire ripening period, then, a dynamic evolution of compounds is a characteristic feature.

Normal Spectrum of Cheese Flavor

The spectrum of major flavors inherent to natural cheese and of commercial significance is clear cut. At one end of the spectrum line is flat flavor and at the other, typical flavor. In between lie a multitude of flavors including acid, bitter, unclean, rancid, fruity, and sulphide. With the exception of typical flavor, all are undesirable in Cheddar cheese. Unfortunately, they are often the predominating flavors.

The Typical Cheese Flavor Sensation

In a negative sense the typical flavor of such a cheese as Cheddar is that flavor which one looks forward to being present in a purchased sample of cheese, but which usually is not. Positively speaking, the typical or characteristic taste of cheese is associated with a pleasant, slightly sweet, aromatic, walnuty sensation without any outstanding single note. This flavor tends to increase in

intensity during mastication of the cheese sample in the mouth. It should not be confused with the harsh, stinging flavor which results from excessive acid in some cheese. In old cheese with typical flavor, a "bitey" quality, being neither coarse nor unpleasant, may prevail giving sharpness to the cheese.

Some cheese affords an illusion of cheese flavor upon being placed in the mouth, but almost immediately thereafter all further sensation of flavor disappears. This "downhill" flavor characteristic relegates cheese of this nature to the flat category.

Among hard rennet cheese, some of the most delicate cheese flavor can be found in good natural French Gruyere and the Swiss Emmental, both of which are associated with a marked sweetness of taste. The true Cheddar flavor is more robust; the Blue and Roquefort more spicy; the Provolone displays marked butyric acid overtones.

Agents of Typical Flavor Formation

Four groups of agents take part in the flavor production of cheese. They are: (a) the natural milk enzymes, ostensively, derived from the blood of the cow; (b) rennet, an added enzyme preparation obtained from ground-up stomachs of young calves; (c) cheese bacteria, either added to the milk as cultures or naturally residing in milk; (d) microbial enzymes. The relative importance of these agents to direct typical flavor production, though never fully established, has been subjected to intense speculation with some accompanying experimentation.

Natural milk enzymes long have been accorded major importance in the development of cheese flavor because of the early discovery by Babcock and Russell *(2)* of an active protease in milk which was named "galactase." This enzyme was thought to hydrolyze the paracasein of cheese into compounds leading to typical flavor. Peterson, Johnson and Price *(17)*, however, since have indicated that natural milk enzymes are not as important in cheese ripening and in flavor production as was previously considered. The potentially important natural protease of milk exhibited optimum activity at pH 8.0, with little or no activity at the initial pH of most ripened cheese, pH 5.0. A somewhat similar situation was found to exist for the natural milk lipase when this enzyme was subjected to a comparable study.

Rennin, the major enzyme of the milk coagulating preparation, rennet, apparently plays no direct part in typical flavor formation. It acts more in an intermediate capacity to produce water insoluble protein substrate from paracasein so that during cheese ripen-

ing other agents can divert this substrate into critical flavor compounds. McKerns *(14)*, in his chromatographic study with rennin, showed that rennin on casein solutions hydrolyzed the molecule only to the peptone level, a level of little individual flavor intensity other than bitter. In 1955 Berridge *(3)* used crystalline rennin for cheesemaking and failed to attain satisfactory cheese flavor. The cheese made with the residual matter of rennet, plus rennin, however, produced good cheese flavor. This is interesting because it shows, as earlier postulated by Kizer *et al. (10)*, that impurities of the enzyme carrier, rennet, may stimulate the desirable microbial fermentation leading to good cheese flavor.

In Cheddar cheese studies, four groups of cheese bacteria have received critical attention for their contribution to cheese flavor.

The lactic acid streptococci, most aptly represented by the organism *Streptococcus lactis,* are important early in the acid production of cheese-making. No strong evidence has been produced to show that they are directly concerned with typical cheese flavor production.

The lactobacilli have displayed characteristics worthy of more serious consideration. One outstanding bacteria of this group is *Lactobacillus casei.* This bacterium, upon its addition to cheese milk, can increase the typical cheese flavor but, unfortunately, bitter side flavors follow in its wake which reduce its practical importance to commercial practice.

A third group of bacteria, the enterococci, has received much scientific attention in cheese technology since 1948. These bacteria are normal inhabitants of the intestinal tract. It was found *(6)* that the addition of freshly isolated enterococci, in this case three mixed strains of *Streptococcus faecalis,* to milk for cheesemaking produced good flavored Cheddar cheese. Since then, another closely related enterococci culture, *S. durans,* has been successfully used in new cheesemaking processes *(22)*. The enterococci are of additional importance to cheese processes because of their high heat and salt tolerance.

Interest in another group of bacteria, the micrococci, has been revived by Alford and Frazier *(1)* who observed that micrococci were normally present in most ripened Cheddar cheese and that the addition of select strains increased cheese flavor, although one strain created a bitter flavor.

These independent studies show then that select, but different, species of bacteria possess the common ability to influence favorably or to increase typical flavor of cheese. This ability is not always exhibited by these bacteria, nor do large numbers of bacteria of this nature in milk necessarily mean a proportional in-

crease in typical flavor intensity. Often the presence of only small numbers of these bacteria in milk leads to good cheese flavor.

The active forces behind this flavor fermentation are enzymes from these microorganisms. In this respect the enzymes may be more important after the microbial cell dies than during its life. In cheese, a few weeks after ripening, many millions of bacteria die with a subsequent autolysis of their cells and a liberation of intact, active enzyme systems. Bacterial protease, peptidase, lipase, decarboxylase, and deaminase are very much in evidence in ripening Cheddar cheese. Environmental conditions are favorable for their activity and many low molecular weight, water-soluble compounds possessing individual flavor are formed. There is now much to support the original thesis of Orla-Jensen *(16)* that microbial enzymes are the direct linkage in the reaction chain leading to typical flavor formation of cheese.

Rate of Typical Cheese Flavor Formation

When typical flavor agents are active in cheese, the rate of flavor development becomes a function of time and temperature. Cheddar cheese is normally ripened at temperatures approximating 40° F. or slightly lower, but may be force-cured at temperatures between 50° to 60° F. Under these circumstances optimum flavor development in pasteurized milk cheese is attained in about 2 months at 60° F., 6 months at 50° F., and 12 months at 40° F. For raw milk cheese a more rapid, but also more variable, rate is the rule.

The relative humidity of the room and moisture content of the cheese exert a direct influence on flavor rate. In addition, salt and acid concentration and the presence or absence of critical amounts of contaminating metals, such as copper, are of importance.

Some Peculiarities of Cheese Flavor

It is of interest to note that following optimum Cheddar flavor development a decline in quality takes place, upon continued ripening or long storage, which is very apparent to flavor judges. This deterioration is relatively slow at low temperatures but often becomes apparent within a month at the higher ripening temperatures or a few days at extremely high storage temperatures. The cheese is criticized as being overripe in flavor.

Though encountered in commercial channels, little is known of the character of this overripe flavor nor, to the knowledge of the

author, has any attempt been made to study its nature. The flavor defies clear-cut description, but obviously the full bloom of the typical cheese flavor is no longer present, even though the flavor sensation is not flat in character.

An interesting question in cheese flavor research is why do not all ripened cheeses display bitter flavors? Cheese is a rich source of proteolytic enzymes, of ideal substrate and suitable pH for extensive proteolysis. Some cheeses are bitter, but bitterness is not inevitable, and the average cheese can be made without bitterness. Cheese, then, represents a delicately controlled fermentation, but the mechanism of this control is still somewhat of a mystery.

Apparently, though, a close association exists between those factors which lead to typical cheese flavor and those which lead to bitter flavor. For example, in the author's laboratory, a cheese made wholly by the addition of a culture of the strongly proteolytic *Streptococcus liquefaciens* resulted in more typical cheese flavor, but this was followed later by strong bitterness. Other instances of the occurrence of the same phenomena with *L. casei* and the micrococci have been cited in this text.

Certain peptides and amino acids play an important role in the production of bitter flavors of cheese. Windlan *(24)* has isolated from Cheddar cheese a polypeptide, containing in its makeup glutamic acid, glycine, proline, valine, alanine, and leucine, directly associated with the intense bitterness of his experimental cheese. One wonders if research cannot be directed at rendering these bitter compounds innocuous in cheese without impairing typical cheese flavor. Perhaps certain bacteria upon addition to cheese can utilize selectively these bitter peptides, or perhaps added enzyme systems can split them to others not possessing the bitter quality.

The Nature of Typical Cheese Flavor

A concept held by some cheese investigators over the years is that, like diacetyl in ripened cream butter, a single compound is responsible for the typical flavor of Cheddar cheese. In 1955 Dacre *(5)* revived this concept by stating it was possible to recover the essential typical flavor principle of cheese through steam distillation. This volatile principle was not any of the fatty acids, nor ethyl alcohol, butyraldehyde, ethyl acetate, or ethyl butyrate, the only substances identifiable in his concentrate. Dacre was not able to identify the individual flavor principle. One might suggest here that the general problem would be a good subject for gas chromatography analysis.

The volatile portion of cheese is well known for its important contribution to cheese flavor and, therefore, it is entirely conceivable that an unknown fraction exists in extremely small concentration possessing typical cheese flavor characteristics. But considerable direct and indirect evidence *(4, 13, 15, 16, 18, 20,* and *22)* has accumulated to show that known components of cheese are significant contributors to typical cheese flavor.

A major characteristic of many ripened cheeses is their heavy concentration of water-soluble end-products. For example, almost half of the cheese protein is converted to soluble nitrogen in aged cheese, and some amino acids of the heavily concentrated casein of cheese are freed to the extent of 10 percent or more of their existing concentrations in this protein. Many of these water-soluble end-products have individual flavor of their own; the amino acids range from bitter and sour to sweet (table 3),

Table 3. Some amino acids existing in ripened cheese and their flavor sensation in pure state[1]

Amino acids	Flavor
Glycine, Alanine, Proline, Serine, Threonine	Sweet.
Valine, Arginine, Histidine, Methionine	Bitter—sweet.
Leucine, Phenylalanine, Tryptophan, Lysine	Bitter.
Aspartic acid	Bitter, sl. broth-like.
Glutamic acid	Broth-like.
Cystine	Rubber-like.
Tyrosine	Almost tasteless.

[1] Flavor data from Mulder, H. *Netherlands Milk and Dairy J., 6,* 157 (1952).

the fatty acids from rancid through goaty. Some peptides have characteristic aroma and flavor. The importance of the presence of the known compounds to the flavor of cheese has been referred to frequently in the literature by many investigators, including Berridge *et al. (3),* Mabbit *(13)* and Virtanen *(22).*

Based on such information, as well as personal observations of their own, Kosikowski and Mocquot *(12),* in a forthcoming volume, have suggested a "Theory of Component Balance" to explain the origin of cheese flavor. In a brief outline here it states that:

1. Typical cheese flavor, particularly in Cheddar and other closely related hard rennet cheese, does not apparently depend upon one key compound but originates from a variety of substances emanating from fat, protein, and lactose.
2. Individually, these components have flavor, but not necessarily cheese flavor. Selectively, when in a critical balance, the resulting flavor is that of typical cheese flavor.
3. Some individual components in this balance are more important than others. Certain fatty acids and amino acids, amines, and peptides together perhaps tend more than any other components to create a taste sensation resembling that of cheese. Other compounds, including esters, alcohols, and lactic acid, exert an important but lesser influence on the typical flavor arrangement.

Evidence for "Theory of Component Balance"

Any theory can be subjected to scientific criticism. The present theory is no exception, yet there exists some substantiating evidence. Mulder *(15)* in Holland has shown the close relationship between typical flavor of Dutch cheese and pure chemical compounds normally associated with this cheese type. Concurrently, research in the United States *(20)* with pure chemical compounds on Cheddar flavor has produced encouraging, although tentative results. Here, the addition to a bland cheese mass of a balanced quantity of pure fatty acids, normally found in cheese, resulted in a distinct butyric-rancid sensation. When pure amino acids and amines, in balance, were added to a second bland cheese mass, a sweet flavor resulted resembling that of Swiss cheese. On a third experiment, the 2 groups of pure compounds were blended with a bland cheese mass, and the presence of a Cheddar-like flavor was indicated by the 3 experienced judges. This flavor was not necessarily complete, but the Cheddar cheese association was definite.

The synthesis of cheese flavor with pure compounds is of basic scientific importance and appears promising. Although experienced flavor judges capable of detecting flavor defects and of estimating the intensity of typical cheese flavor participated in this investigation, a knowledge of the character of typical cheese flavor of various ripened cheese might well be enriched by the inclusion of "Flavor Profile" studies. This point now is being considered.

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Atypical Flavor of Cheese

A slight shift in the proportion of these compounds in cheese, through changes in ripening techniques or micro-organisms, may, without destroying typical cheese flavor, lead to a change in its quality. A strong shift may, on the other hand, lead to atypical flavor. Both of these movements are tenable in the "Theory of Component Balance."

In cheese the natural forces at play apparently can be diverted easily into channels resulting in an overbalance of components. Such atypical flavors as bitter, rancid, unclean, and sulfide are dependent to a great extent on the intensity and direction of the fermentation inherent in cheese ripening.

Rancid flavor of cheese is closely related to an overbalance of lower chained fatty acids, particularly butyric. Unclean flavor, as pointed out by Hylinka and associates *(9)* is closely associated with intense rancid flavors. Recently *(21)* it was observed that the occurrence of unclean flavor is accompanied by increased concentrations of γ-amino-butyric acid, even though the latter compound may not be the causative factor. Sulfide flavor, now much in evidence in block Cheddar, results from an overproduction of hydrogen sulphide by the action of bacterial enzymes from the lactobacilli *(19)* and perhaps other species.

Cheese flavor development is a result of a controlled fermentation, but jiggle the controls a little and atypicalness results.

Research Underway

Present flavor studies on cheese underway in laboratories throughout the world, with some exceptions, are concerned with correlating the presence of compounds to cheese flavor. This represents a more-or-less deductive approach to cheese flavor research.

At Cornell University, research now in progress has taken on a more inductive character. Research on the creation of typical flavor in natural cheese through addition of bacterial enzyme systems to green cheese is underway.

The bacterial enzyme project is of interest because of its scope. At the moment 15 different species of microorganisms have been grown, separately, in 60-gallon lots of fresh Cheddar cheese whey buffered at 6.5–7.0. The bacteria are grown under conditions which it is hoped will result in a maximum production of certain enzymes within their cells. Following this, centrifugation, using

a large cream separator, is employed to remove the bacteria from the whey. Then the cells are disrupted using either an acetone-ether method employed by Gale *(8)* or the gas pressure chamber method of Frazer *(7)*. The active cell enzymes liberated from the dead bacterial cells are harvested and reintroduced into cheese. This project should indicate what bacterial enzymes, alone or in combination, can induce typical flavor into cheese and the degree of intensity of resulting flavor.

As more knowledge of the basic fermentation patterns of cheese is vital to such inductive research, an accompanying long-range project at Cornell University, in cooperation with the University of California, has been started in the field of isotopic labeling of cheese milk and cheese. The individual components of milk and cheese are to be labelled with C^{14} and reintroduced into cheese milk. Then the nature of the resulting end-products during ripening can be more closely defined and the specific effect of bacteria or bacterial enzymes more clearly appraised.

Summary

Time limitation, naturally, prevents the full development of problems and research dealing with the broad field of cheese flavor. Many general problems are apparent. One of basic scientific interest and perhaps of potential commercial importance is that concerned with the seasonal variation of typical cheese flavor. In the United States why does milk obtained from the cow in May, June, or July produce cheese of more typical flavor than in any other month of the year? Why will cheese buyers pay significantly more for this cheese? In Europe for Gruyere cheese a similar phenomenon occurs, not during this period but in October and November. It would be a worthwhile achievement to obtain even a minor lead as to the cause of this phenomenon.

Another general problem resides in the realm of pasteurized milk cheese and raw milk cheese. Significant differences in flavor are apparent, with the raw milk cheese flavor usually being preferred. The pasteurized milk cheese often is referred to as being incomplete in flavor. Because of the safety factor involved, about 80 percent of Cheddar cheese of the country is made from pasteurized milk. An objective in the field of cheese technology has been to reproduce this exact flavor of raw milk cheese in the pasteurized milk product, but without success. Here again a hampering factor is the lack of knowledge concerning the chemical differences between these 2 types of cheese. However, some

progress has been made, as indicated earlier, and more can be expected.

Marked interest in the field of cheese flavor research has been evidenced since 1895 by the efforts of such eminent scientists as Von Freudenrich, L. L. Van Slyke, Orla-Jensen, J. M. Sherman, A. I. Virtanen and many others. Now that adequate tools are available, progress can only be limited to the range of imagination of the scientists active in this field.

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Bread Flavor

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It is probably a truism to point out that in bland food products, sensory judgments will be influenced heavily by tactual characteristics. White bread is ordinarily rather bland in odor and taste, so that flavor comparisons, even by a trained test panel, will in all probability reflect in some degree the crumb textures of the various samples. There is no unanimity of opinion as to what flavor properties are the most desirable ones in bread. Preferences vary widely between regions as well as countries, both as to flavor and texture. It is not surprising, therefore, that progress in the investigation of bread flavor factors has been slow. Even the magnitude of the part played by flavor in bread acceptance is in considerable doubt. Many people are highly critical of the blandness of currently-sold white bread, especially those who are old enough to recall the homemade bread which was fairly common several decades ago. Some few still have access to home-baked bread or rolls, while there is a small but loyal market for a number of brands of home-style, compact loaves which command premium prices. However, the fact remains that a good 90 percent of all bread sold is the conventional type of white bread. An extensive consumer preference study conducted in Rockford, Illinois recently *(12)* demonstrated clearly that, of several types of bread supplied, the type preferred was essentially identical with most white bread produced by the large wholesale bakeries at present.

To what extent is flavor a factor in bread preference and consumption? An idea of the state of thinking with regard to bread flavor may be gained from the results of the above-cited study. Homemakers who took part in the bread consumption studies also were canvassed for their views on the following:

What bread characteristics would they, as judges, be most critical of?

What bread characteristics would they consider as most desirable?

What were their opinions about white bread as currently available in supermarkets, etc?

More than half named "poor taste" as an important factor for criticism, and about half considered "good taste" as important to quality. However, among the opinions of the typical commercial product, only about 15 percent cited its good flavor, and an equal

percentage cited its poor flavor or lack of flavor. It would appear that the average homemaker, at least in this sampling, professes greater discrimination than she practices.

As with all processed foods, the flavor of bread is a function partly of the ingredients and partly of the processing. The normal ingredients of bread, apart from certain specialty breads containing caraway seeds, raisins, cheese, or other such additions, are all mild in flavor, as is a freshly mixed dough. The tremendous complex of enzymatic reactions during fermentation gives rise to the formation of many new substances sufficiently volatile to produce olfactory stimuli. The actual baking process, in the course of which crust is formed at a temperature which may reach 150° C., while the loaf interior approaches the boiling temperature of water, engenders many new reaction products which contribute greatly to flavor.

Role of Ingredients

Flour as such contributes very little to flavor, assuming of course that the flour is free of foreign matter, such as fumigant residues, and was milled from sound wheat. Wheat germ, as found in whole wheat flour, has a characteristic nutty taste and aroma, but this is extremely diluted in the actual flour. In a taste panel comparison of breads baked from white flour and wheat germ flour, the test was conducted in a darkroom illuminated only by yellow light, masking the difference in crumb color. Panel members were unable to distinguish between the two breads with any degree of statistical significance *(1)*.

Yeast has a characteristic flavor, but the amounts normally used in breadmaking will not contribute significantly to the bread flavor. Baking-powder biscuits to which 3 percent of compressed bakers' yeast had been added when mixing the ingredients, and which were baked immediately, could not be distinguished in flavor from biscuits made identically, but without adding yeast *(1)*.

Milk, usually in the form of nonfat roller- or spray-dried milk solids, is an important minor ingredient in much of the white bread produced today. From 3 to 6 percent, expressed on a flour-weight basis, is the common range for nonfat milk solids. Oxidized flavors in the milk solids may be detectable in the bread; but a more serious danger is that oxidation products from the milk solids may react with the lipids of the flour, yeast, and shortening to produce additional objectionable flavors.

Ingredients such as malt and "yeast foods" are used in proportions too small to affect the flavor of the finished product mate-

rially. Added sucrose is largely used up during fermentation, but when added at fairly high levels, there is a discernible difference in taste (due in large part to the sweetness of fructose). Salt, although used at low levels (about 2 percent based on flour weight is usual), has a vital part in enhancing the sensory appreciation of any baked product. Commercial bakery shortenings as used today, made mainly from highly refined and deodorized fats, show excellent stability at oven temperatures; a good antioxidant-containing shortening should not contribute off-flavors.

Role of Fermentation

The chief end-products of panary fermentation are ethyl alcohol and carbon dioxide. Many other compounds are formed enzymatically which have definite flavor characteristics. It is possible to bake bread from a yeasted dough which is not given time to ferment but is raised by chemical gas production; such bread will look and feel acceptable, but will be deficient in taste and aroma *(4)*. On the other hand, some commercially-practical processes for breadmaking carry out fermentation separately from the flour, and yield bread of excellent flavor. One such method, the American Dry Milk Institute Stable Ferment method, consists in allowing yeast to ferment sucrose in a solution, containing also salt, milk solids, yeast food, and perhaps malt. The liquid ferment then is added to flour, a dough is mixed, and this is immediately ready for moulding, proofing, and baking. Bread baked by this technique was compared by a trained panel with bread baked by a conventional sponge-and-dough method from the same ingredients; there was no difficulty in distinguishing the two products by flavor, but no significant preference was shown for one over the other *(2)*.

Comparing bread baked by the sponge-and-dough method with that baked by the straight-dough method, it is generally considered that the latter develops better flavor from the same ingredients. Although there is some evidence that the physical treatment in mixing has something to do with flavor in bread, the differences in fermentation are probably chiefly responsible for the flavor differences between the two types of bread. Bread baked from hand-moulded doughs has been reported to be superior in flavor to that from machined doughs *(6)*, and it may be that the greater physical abuse caused by machining allows more of the volatile flavorants to escape from the gas cells in the dough before oven heat can partially seal these cells.

As to the types of substances which may be formed during fermentation, most of our information has been provided by the brewing and winemaking scientists. Luers *(11)* has reviewed some of the mechanisms whereby keto acids, aldehydes, and higher alcohols may be produced from amino acid substrates by enzymatic reactions, while intermediate aldehydes can undergo the acyloin synthesis to yield carbinols, and dismutation to yield acids and alcohols. Additionally, the tricarboxylic acid cycle contributes a number of other organic acids, while under favorable conditions esterification of acids with alcohols may occur.

From this multiplicity of biochemical and chemical changes, a large number of flavorants are formed. Many of these, however, are probably too volatile to remain in bread after baking, except in trace amounts. Volatile substances can be condensed out of oven vapors; Baker and associates *(3, 5)* have investigated these oven condensates, and have detected a variety of flavorants. How representative the oven-volatile substances are of those substances which remain in the bread is highly problematical, however. Undoubtedly, too, some of the substances produced during fermentation undergo further reactions at oven temperatures. Thus it becomes difficult to establish unequivocally the origin of any compound which is detected in bread or in the oven gases.

Role of Oven Reactions

How important oven reactions are in producing flavor compounds will be apparent to anyone who has tasted fermented dough. It is possible to cook bread by passing through the dough an alternating current which acts as a resistor and allows considerable heat to be developed *(3)*. Bread made in this way has no crust, and only a very mild, yeasty flavor. Clearly the conditions necessary for the formation of a browned crust are equally essential to the development of full, appetizing bread flavor. That reducing sugars must be implicated in the production of flavor as well as of crust color was demonstrated by Baker *(5)*, who compared bread baked from the same dough after normal fermentation and after excessive fermentation. The latter, in which all free reducing sugars had been used up by the yeast, yielded bread resembling that made by electric resistance heating, in its lack of either normal color or flavor. It is reasonable, then, to presume that Maillard-type reactions give rise to flavorants as well as to colored products in normal bread crust.

Whether appreciable amounts of volatile compounds are formed by purely pyrolytic reactions during baking is not certain. Al-

though caramelization does occur at the crust surface, the actual crust temperature attained in an oven operated at 450° F. (232° C.) does not exceed about 150° C. The interior of the loaf approaches to within a degree or two of the boiling point of water, but does not quite attain it, during a normal baking period. Under these conditions, it is likely that pyrolytic breakdown of reducing sugars will be of much less consequence than Maillard-type condensations between reducing sugars and amino compounds, leading to flavor compounds by subsequent decompositions.

A modest amount of literature exists on the production of aromatic substances by heating together single amino acids and single reducing sugars. Lea *(10)* has reviewed some of this work, and has emphasized the very high temperature coefficients of these condensations. Information gained from such simple model systems may prove helpful in elucidating the origins of thermally-produced flavor compounds; but even the reaction between glycine and glucose has yielded more than 2 dozen compounds, few of them characterized *(7)*. Hence the study of flavor substances produced via Maillard reactions probably will remain a difficult field.

Some Flavor Compounds Detected in Bread

Other than the previously cited work of Baker *et al. (5)*, the literature records few compounds actually identified in bread which can be considered flavorants. Volatile aliphatic acids (acetic, propionic, etc.) obviously are implicated, especially in sour rye and similar breads; these products also contain significant amounts of furfural and volatile esters *(9)*. Acetoin, the oxidation of which yields diacetyl, a potent flavorant, has been found in varying concentrations in white bread *(13)*; acetoin is said to be a fermentation by-product

In studies carried out in the laboratories of the American Institute of Baking, several nonvolatile organic acids, as well as their ethyl esters, have been identified in the crumb of fresh bread. These compounds were solvent–recovered from aqueous extracts of bread crumb, and were isolated by column partition chromatography. The quantities estimated titrimetrically may not represent the total concentrations of these acids and esters in the crumb, due to differences in extractability. Succinic, lactic, and itaconic acids were not unexpected, since all are intermediates in carbohydrate metabolism. Hydrocinnamic and benzilic acids also were found; these are not readily explainable. Storage experi-

ments were performed in which double-wax-wrapped loaves were held at 70° F. and at —20° F. Preformed and ester acids were resolved and estimated by titration in the crumb extracts of loaves after various times. Seven days of storage at 70° F. produced no discernible decreases in the amounts of the preformed acids, while ester acids were depleted steadily, as expected. At —20° F. ester acids again disappeared more or less uniformly over 86 days, while an unexpected result was the progressive accumulation of preformed acids at this temperature. Succinic acid in fact showed an increase of ninefold over the course of the study. Such large increases might be accounted for if the esters were depleted by hydrolysis rather than volatilization, or if the organic acids were liberated gradually from unextractable complexes. Further investigation of the quantitative aspects is underway *(14)*

Current studies at the American Institute of Baking are concerned with carbonyl compounds occurring in the crust of freshly-baked white bread. Steam-volatile carbonyl compounds have been converted into their 2,4-dinitrophenylhydrazones, which are being resolved and identified by paper chromatography. Compounds already identified include furfural, diacetyl, puruvic acid, ethyl puruvate, acetaldehyde, levulinic acid, and ethyl levulinate. The ethyl esters may be artifacts arising due to the use of aqueous ethanol as the crust extraction medium. All of these compounds except levulinic acid have pronounced odors, and undoubtedly are important contributors to the aroma of bread.

In experiments preliminary to the studies on carbonyl compounds from crust, it was noted that a freshly-prepared, filtered crust extract, using water as the extractant, possessed a rather bitter taste in addition to a fine breadlike aroma. After steam distillation, the undistilled material retained more of the pleasant aroma than distilled over, while the bitter principle had passed into the distillate. Not all of the odorants in the distillate were carbonyl compounds; after separation of the hydrazones, there was still some aromatic material, whose character was weakened and altered, but not eliminated, by alkali saponification. Thus it appears that the crust extracts contain a variety of odorants besides carbonyl compounds, esters, and acids. A further observation was that the aromatic principles in the undistillable extract could be segregated almost completely by ether extraction. Pigmented products of crust browning remained in the aqueous phase, where they precipitated slowly; this ether-stripped aqueous phase was practically devoid of odor, and had only a faintly sweet taste. It is planned to examine the composition of the ether-soluble material shortly. Quantitative estimations of the carbonyl com-

pounds can be made spectrophotometrically at very low concentrations *(8)*.

Unquestionably one of the most powerful tools to become available to the investigator of food flavors is gas chromatography. Using this technique, it has become possible to separate and identify extremely small amounts of moderately volatile substances without conversion into derivatives. At least one laboratory is currently engaged in a study of the flavorants recoverable from the vapors of cooling bread, by adsorption of the vapors on activated charcoal and subsequent extraction with a volatile solvent.

Due to the extremely reactive and unstable natures of some types of compounds which are likely to be implicated in flavors, their detection and isolation in the "native" form may prove difficult. Solvent extraction with alcohols may lead to esterification of organic acids, while conversely, esters may be destroyed by hydrolysis in alkaline or acid extraction media. Aldehydes in general are highly susceptible to oxidation and polymerization, as well as to Maillard-type condensations. It can be appreciated, then, that rapid and sensitive physical methods, of which gas chromatography is one of the most striking new examples, are mandatory to the success of continued research into the nicer subtleties of food flavors.

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Study on Development of Beef Flavor in U. S. Choice and U. S. Commercial Cuts of Sirloin[a]

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The purpose of this paper is to define beef steak flavor and to trace its development under commercial practices of aging or ripening. The findings which were obtained through a flavor profile panel might be regarded as the first step in attacking the chemistry of beef flavor; hence, their presentation at this conference on the chemistry of natural food flavors. Moreover, it should be useful and interesting to have organized information from which to draw possible reasons why beef is such a popular meat in the United States diet.

The meat for this project was steer meat bought through a local wholesaler at the slaughter house (kosher). At desired intervals, steaks 1 to 1½ inches thick were cut for us from the loin beginning at the 13th rib and progressing toward the round. Use of the loin gave several advantages: (1) the meat is relatively uniform from steak to steak; (2) size of each steak would permit adequate test portions for a 4–5 membered flavor panel; (3) approximately 8–12 steaks, depending on the size of the animal, would be available; and (4) steaks are most often broiled, and this method of preparation could be most readily standardized and controlled.

Following is an outline of slaughter and holding procedures necessary for the understanding of terms used to describe the age of beef:

(1) After slaughter, bleeding, decapitation, washing, slitting, removal of the hooves, hide, and various organs, cutting is completed. Kosher seals and stamps are applied. The meat at this point is called "hot."

(2) The carcass then is washed and shrouded; the shrouded sides are removed to a chilling room equipped to maintain an average temperature of 33–34° F. The next day the sides are removed to a second chilling room where they are unshrouded, quartered, and sold to a wholesaler. At the wholesale house, the meat is Government-

[a] Part of a study sponsored by International Minerals and Chemical Corporation in 1955. Helpful technical advice was given by Mr. C. F. Evers and Mr. Louis G. Buettner of Ac'cent-International.

graded and hung in humidity-controlled refrigerators at 36–38° F. This is "green meat" until it is aged. We were told that the usual aging period for U. S. Choice quality is 12 days.

Table 1. List of studies for beef flavor project

Study	Meats	Cuts
1. Flavor panorama	Aged beef, veal, pork, and lamb	Comparable cuts (loin).
2. Flavor and tenderness	U. S. Choice, aged Western steer.	1 loin.
3. Flavor and tenderness	U. S. Choice, green to aged (off bone) Eastern steer (2 days to 10 days).	1 loin.
4. Flavor and tenderness	U. S. Choice, hot to green (on bone) Eastern steer (3 hours to 51 hours).	Right loin, 506 lb. carcass.
5. Flavor and tenderness	U. S. Choice, hot to aged (on bone) Eastern steer (3 hours to 15 days).	2 loins and 2 tenderloins, 720 lb. carcass.
6. Flavor and tenderness	U. S. Commercial, hot to aged (on bone) Eastern steer (3 hours to 15 days).	2 loins, 675 lb. carcass.

Table 1 is the chronological listing of studies reported in this paper. About 50 flavor panel sessions were held to obtain the findings. The first two studies served to orient the panel to meat flavor in general and to beef flavor in particular. The next 3 studies were devoted to U. S. Choice loin steaks ranging in age from 3 hours to 15 days after slaughter. Study 5 included tenderloins as well. The last study was concerned with U. S. Commercial steaks, age 3 hours to 15 days.

The preparation of all the beef samples was made by a home economist. The steaks were medium-broiled, 3 inches from the source of heat for 6 minutes on each side in a preheated electric oven. They were carved immediately at the testing table and served on warmed china plates to the panel members who already were seated and waiting. Using flavor profile procedure, the panel examined aroma first and then flavor-by-mouth. Attention also was given to tenderness.

Profile procedure uses standardized procedures and flavor-oriented panel members who then train themselves to analyze the aroma and flavor of the product under study. The results of their study is a tabulation of the detectable aroma and flavor characteristics noted separately by descriptive terms, listed in the order perceived and quantified as to intensity as follows:

0—not detected.
) (—recognition threshold.
1—slight.
2—moderate.
3—strong.

In addition, overall impressions of the aroma and of the flavor—called amplitudes—also are considered and evaluated. Amplitudes can range from very low through low, moderate, and high —) (, 1, 2, and 3, respectively.

Panorama of Meat Flavors

Since mainly sirloin steaks were to be studied for the beef work, retail cuts as close as possible to sirloin steaks were obtained of high quality veal, pork, and lamb. The various "steaks" were trimmed and then broiled and examined in separate panel sessions. A brief summary of the findings is given in table 2.

Table 2. Panorama of meat flavors

Beef loins 1½ in. thick	Veal cutlets ¾ in. thick	Pork chops 2 in. thick	Lamb steak 1½ in. thick
Major Aroma Characteristics			
Animal (liver). Brothy. Nosefilling. Sweet.	Animal (liver). Sweet.	Sweet. Fatty Animal (sulfide).	Fatty acid. Animal (liver). Tallow. Sour.
Major Flavor Characteristics			
Serum. Brothy. Animal (liver). Mouthfilling. Salivating. Bouquet.	Sweet. Animal (liver). Sour.	Sweet. Animal (chicken). Mouth coating. Sl. salivating. Dry.	Sour (fatty acid). Animal (liver). Tallow. V. sl. salivating.
Aftertaste			
Nosefilling. Salivating. Mouth satisfaction.	Not noted.	Mouth coating	Greasy-coating.
Remarks			
High amplitude. Interesting.	Unblended. Flat.	Bland.	Dominated by animal note.

Beef. In the aroma, the animal note was found to be stronger from within the meat than from the surface. The other three

characteristics listed in the table emanated from the cooked surface. Brothiness is typical of beef and includes caramelized- and bouillon-type factors. Nosefilling is a sensation due in part to brothiness and to the broiled fat. Sweet is best defined as the sweet character associated with nuts—it is not floral nor is it sugary.

As beef is chewed, one of the early-perceived taste factors is serum—a combination of blood salts and salivation. The serum undoubtedly is attributable to the uncooked center stratum of the steak. (Early in the program, it was found that the fullest flavor spectrum was obtained from a "medium" broil. Rare steaks emphasize animal and serum; well-done steaks lack serum effects and are relatively dry.)

Another important flavor factors is the broth note which may be thought of as cooked serum plus other factors of pyrolysis. It is entirely compatible with the serum note. The animal character in the flavor resembles the animal note in liver; but in fully aged, Choice-grade sirloin the animal note is usually of low intensity. (Later, we noted that tenderloin cuts were prone to have a higher intensity of animal factor.) Mouthfulness is a well-recognized factor of beef-steak flavor and in part derives from salivation. The so-called bouquet listed as a flavor characteristic of the beef refers to the sensation of full flavor perceived each time the meat is chewed; a better description would have been "flavor bloom," but, since the meat industry's vocabulary contains the word "bloom," we have avoided its use in connection with beef flavor.

The mouthfulness of beef lasts into the aftertaste as do salivation and aromatic factors, summarized as nosefilling. All of these apparently contribute to the goodness of flavor; moreover, at the levels perceived they are non-satiating.

Veal. Since veal comes from the same species of animal as beef, one would expect it to have some characteristics in common with beef. The panel found the greatest similarity was in the animal factor of both flavor and aroma. Amplitudes for the aroma and flavor of veal are considerably less than for beef. In fact, the flavor is considered flat except for the predominance of the animal note. Such factors as mouthfulness, salivation, brothy, and bouquet are almost entirely missing. It is possible that because of its lack of such flavor factor, veal cutlets are breaded, fried, and frequently served with tomato-base sauce. Such treatments would add flavor interest and balance.

Pork. The aroma of broiled pork chops also was of lower intensity than that of steak. Predominant were the sweet and fatty

notes. The animal character was not aminelike but rather sulfide, like chicken. The flavor was even more chickenlike, with sweetness prominent. Although some salivation was noted in the flavor, the meat itself was not juicy. Mouth coating was perceived. In contrast to the veal, pork was characterized as bland rather than flat.

Lamb. Broiled lamb steak in both aroma and flavor is predominantly animal—fatty acid, tallowy, and amine (liver). There is no balance of flavor in lamb; its aromatic factors far outweigh the taste and feeling factors.

It might be conjectured that the frequent serving of mint jelly —that is a strong sweet taste plus a strong aromatic character— is to overwhelm the animal note and to divert the attention of the eater.

U. S. Choice Sirloin

Table 3. Effects of aging on aroma and flavor of broiled U. S. choice sirloin (carcass weight—506 lb.)

	Hot meat			Green meat	
Age in hours	2	3½	24	27	51
Aroma Notes and Their Intensities					
Amplitude	)(	)(	1	1	1–2
Animal	1	2–3	2–3	2–3	2
Brothy	0	0	1	1	1–2
Nosefilling	0	0	0	0	0
Sweet	0	0	0	0	0
Sour	0	1	2	2	2
Fatty	0	0	1	)(	0
Bloody	1	?	1	)(	0
Flavor Notes and Their Intensities					
Amplitude	)(	)(—1	1	1 (MSG)	1–2
Sweet	0	0	0	1	1–2
Serum	0	1	1	1	1
Brothy	0	0	0	0	)(
Animal	)(	)(	)(	)(	)(
Mouthfilling	0	)(	1	1	1–2
Salivating	0	0	0	0	)(—1
Bouquet	0	0	0	0	0
Sour	2–3	2–3	2–3	2–3	2
Metallic (Met)	+	+	+	+	+
Astringent	+	+	+	+	+
Aftertaste					
	Soapy. Met. Not steak.	Same.	Sour. Met. Not steak.	Sour. Met.	Met. Sour.

Table 4. Effects of aging on aroma and flavor of broiled U. S. choice green sirloin (boned out at 27 hours)

Age in hours	48	54	72	96	168	240
Aroma Notes and Their Intensities						
Amplitude	)(—1	1—	1	1–2	1–2	2
Animal	2	3	2	1–2	1–2	1
Brothy	0	)(—1	1	1	1–2	2
Nosefilling (N)	0	0	)(	)(—1	1	2
Sweet	0	0	0	)(—1	1	1
Sour	3	2	2	1	1	1
Fatty	2	1	)(	0	0	0
Flavor Notes and Their Intensities						
Amplitude	)(—1	1—	1–2	2—	2—	2
Sweet	0	0	)(	1	1	1
Serum	0	0	0	1	1	1–2
Brothy	0	0	)(—1	)(—1	1–2	2
Animal	1	2–3	2	1–2	1	)(—1
Mouthfilling (M)	0	)(	)(	1	2	1–2
Salivating (Sal)	0	0	)(—1	1	1	1–2
Bouquet	0	0	0	)(—1	1	2
Sour	2–3	2	2	1 (late)	1	)(
Metallic (Met)	+	+	+	0	0	0
Astringent (Astr)	+	+	+	0	0	0
Aftertaste						
	Astr. Met. Flat.	Astr. Met.	Astr. Met.	M Sal.	M Sal. N	M Sal. N

Tables 3 and 4 summarize the panel findings for the aroma and flavor of hot and green sirloin. The hot samples reviewed in table 3 were taken before chilling and held at room temperature the designated number of hours. The green meat was taken at specified times from the chilled carcass. For the samples in table 4, the strip loin had been removed from the chilled carcass 27 hours after slaughter and held in the wholesale house refrigerator. Thus, the steaks tested had not been held on the bone.

The hot meat had almost no recognizable beef flavor. It was rather sour, quite metallic, and quite astringent. Tenderness was lacking, and the experience of eating broiled hot meat was rather unpleasant.

The first two samples listed in table 3 were taken before rigor mortis had begun; the meat, though firm, was very difficult to cut, and its fatty covering was mobile and separate from the flesh.

Broiled meat which had been retained on the bone 24 hours after slaughter was better appearing, and tasted and smelled

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slightly more like beef than did the hot meat. A sweet-salty taste similar to that of monosodium glutamate was detected in such meat. Other notes also had developed: mouth-filling factor was one and serum another. An additional day of aging showed considerable improvement in beef flavor development. Brothy and serum notes were found, and saliva-stimulation was noted. However, the flavor was still metallic and astringent, and the bouquet had not developed.

In table 4, the characteristics for meat aged off the bone are noted. At 48 and 51 hours, the flavor of such meat was not as well developed as that of 51-hour beef aged on the bone. The 96-hour steak was fairly comparable to the 51-hour aged-on-the-bone steak—a matter of almost 2 days.

Table 5. Effects of aging on broiled U. S. choice sirloin —left loin (carcass weight—702 lb.)

	Hot meat			Green meat		
Age in hours	3	6	24	48	96	192
Steak No.	2	3	5	7	9	11
Aroma Notes and Their Intensities						
Amplitude	2	2	2	2+	2	2
Animal	1	1	1	1–2	1–2	1
Brothy	2	1	2	2+	2	2
Nosefilling (N)	0	0	)(	)(—1	1	1
Sweet	0	1	1	1	1	1
Sour	1–2	1	)(—1	1	1	1
Fatty	1	0	0	0	0	0
Metallic (Met)	+	0	0	0	0	0
Flavor Notes and Their Intensities						
Amplitude	1	1+	2	2	2	2–3
Sweet	1	1	1–2	2	2	1
Serum	2	2	2	1–2	1–2	1–2
Brothy (B)	0	0	)(	1–2	2	2
Animal	1	)(	)(	1–2	1–2	2
Mouthfilling (M)	1	1	1	1–2	1	1
Salivating (Sal)	0	)(	1–	2	2	2
Bouquet	)(	0	1	1–2	2	2
Sour	1	1	1–2	1	)(—1	)(
Metallic (Met)	high	high	0	0	+	0
Astringent (Astr)	high	high	0	0	0	0
Nosefilling (N)	0	+	0	)(—1	1	1
Aftertaste						
	Flat.	Met. Astr. Fatty.	B	N	Sal. N	N Sour.

Table 6. Effects of aging on broiled U. S. choice sirloin —right loin (carcass weight—702 lb.)

	Green Meat				
Age in hours	24	48	72	168	336
Steak No.	1	3	6	8	10
Aroma Notes and Their Intensities					
Amplitude	2	2	2	2	2–3
Animal	1	2	1–2	1	1
Brothy	1	)(—1	2	2	2
Nosefilling (N)	)(—1	)(—1	1	1	2
Sweet	0	)(	)(	1	1
Sour	—	1	1	1	2
Flavor Notes and Their Intensities					
Amplitude	1–2	1	2	1	2–3
Sweet	1	1	)(—1	)(	1
Serum	2	—	)(—1	)(—1	2
Brothy	)(	)(—1	2	1	2
Animal	)(—1	1	1	)(	2
Mouthfilling (M)	1–2	1	1	1	2
Salivating (Sal)	2	1	2	1	2
Bouquet	)(—1	1	1	1	2
Sour	2	1	1	1–2	0
Metallic (Met)	high	high	+	+	0
Astringent (Astr)	high	high	0	0	0
Aftertaste					
	Sour. Met.	Sal.	N	Sour. Met.	Sal. N

Tables 5 and 6 summarize the panel findings for steaks cut from the right and left loins of a U. S. Choice grade steer. As for the preceding studies, the hot meat was taken right after slaughter, before chilling. It was held at room temperature until tested. The green meat was taken after the carcass had been chilled for the hours specified in the table. The steaks were numbered, starting from the rib section and proceeding toward the round.

The same flavor and aroma trends were noted for the sirloin steaks taken from the 720-pound steer as from the 506-pound steer, although the heavier steer showed better flavor characteristics at each comparable time period. As hot meat, the broiled steaks were not pleasant tasting, being predominantly metallic and astringent. Not until the hot meat had been held 24 hours at room temperature did salivation and bouquet, so typical of steak flavor, show any significant intensities. By this time, the

sour and metallic character notes had disappeared. This statement pertains to the left loin steaks, but not to those from the right loin which were sour and metallic even as green meat.

From time to time differences were noted between the right and left loins. The general impression of the panel during this particular study was that the left loin was superior in flavor and tenderness. However, not much emphasis is given to this finding since it resulted from a single study. Incidentally, we were told by the wholesaler that ordinarily the right loin is the superior one.

The question arises as to whether there are flavor differences between steaks taken from the extreme ends of a loin. No answer is available from this project. We were told that there is a tendency for the better-flavored meat to be found near the ribs.

Returning to the effects of aging—it is noted that although a full flavor-by-mouth develops by the eighth day (192 hours), sourness is present in the aftertaste. A fully aged steak would have salivation, mouthfulness (mouth satisfaction), and nosefulness as the aftertaste.

As the steaks became more tender, the bouquet developed. Undoubtedly, chewability is partly responsible for the repeated impact of steak flavor (bouquet). But there must be additional factors as well.

The panel confirmed E. C. Crocker's *(1)* findings that the taste and feeling factors (sweet, sour, serum, salivation, mouthfulness) reside in the serum or juices; the fibers seem to produce brothiness, animal character, nosefulness, and bouquet.

U. S. Commercial Sirloin

Table 7. Effects of aging on broiled U. S. commercial sirloin —right loin (carcass weight—675 lb.)

	Hot meat		Green meat		
Age in hours	3	21	46	144	192
Steak No.	2	3	5	7	9
Aroma Notes and Their Intensities					
Amplitude	)(	)(	)(—1	1–2	1–2
Animal	2	2–3	1	1–2	1–2
Brothy	0	0	)(—1	1	1
Nosefilling	0	0	0	0	)(—1
Sour	2	2	0	0	0
Fatty	2	2	2	)(—1	)(
Metallic	0	0	0	0	0
Corn Amine	2	1	1	0	0
Bloody	0	1	0	0	0

Table 7. Effects of aging on broiled U. S. commercial sirloin—right loin (carcass weight—675 lb.)—Continued

	Hot meat		Green meat		
Age in hours	3	21	46	144	192
Steak No.	2	3	5	7	9
Flavor Notes and Their Intensities					
Amplitude	0	)(	)(—1	1	1–2
Serum	0	)(—1	1	1	1
Brothy	0	0	)(	0	)(
Animal	2–3	2	1–2	2	2
Mouthfilling	0	0	0	)(	1
Salivating (Sal)	0	)(	)(—1	)(—1	1
Bouquet	0	0	)(	0	)(
Sour	2	2	1–2	1–2	1–2
Metallic (Met)	—	+	+	+	—
Astringent	—	+	+	+	—
Fatty-soapy (S)	2	2	1	1	1
Fishy	—	—	—	—	—
Aftertaste					
	Off.	Greasy.	S	S	S Met.

Table 8. Effects of aging on broiled U. S. commercial sirloin —left loin (carcass weight—675 lb.)

	Green Meat			
Age in hours	21	72	168	336
Steak No.	2	3	5	7
Aroma Notes and Their Intensities				
Amplitude	)(—1	1	1–2	2—
Animal	2	2	2	2
Brothy	0	)(—1	1	1
Nosefilling	0	0	)(—1	1
Sour	2	1–2	1–2	1–2
Fatty	2	1	1	—
Corn Amine	0	1	0	0
Flavor Notes and Their Intensities				
Amplitude	)(—1	0	)(—1	1+
Serum	—	0	1	1
Brothy	0	0	0	)(
Animal	2	2–3	2–3	2–3
Mouthfilling	0	0	0	)(
Salivating	0	0	0	)(—1
Sour	2	2–3	2	2
Metallic (Met)	+	+	+	+

Table 8. Effects of aging on broiled U. S. commercial sirloin—left loin (carcass weight—675 lb.)—Continued

	Green Meat			
Age in hours	21	72	168	336
Steak No.	2	3	5	7
Astringent	+	+	+	+
Fatty-soapy (S)	2	—	2	1
Fishy	?	?	—	—
Aftertaste				
	S Greasy.	Met. Off.	Met. Greasy.	Sour. Greasy.

Tables 7 and 8 give the panel findings for the right and left sirloins. Hot meat was taken from the right loin and tested 3 hours and 21 hours after slaughter. The 21-hour sample had been held at room temperature. All the other steaks were taken after the carcass had been chilled; these steaks were aged on the bone.

The 3-hour steaks were not recognizably beef by flavor or aroma. The presence of a note found in canned sweet corn (corn amine) was not appropriate. A zero amplitude rating was assigned to the flavor. The predominant flavor notes were animal, sour, and soapy. The serum note and salivation did not appear until the 21-hour sample, which also tasted metallic and astringent. The hot meats were very dry and crumbly. In fact, texture of the Commercial sirloins throughout the study was never quite right—the meat always lacked succulence and crumbled when it was chewed.

The steaks taken after chilling, but only 21 hours after slaughter, were a little more beef-like in flavor than 21-hour hot steaks. This result could be attributable to (1) holding on the bone; or (2) the fact that the steaks had been taken from different loins. However, it should not be construed that the 21-hour green steaks were acceptable in flavor or aroma. Off-notes described as fatty-soapy and fishy were present in both the flavor and aftertaste of even the 72-hour steaks, and the entire flavor was unblended, distracting, and highly unsatisfactory.

During the 14-day aging period, the meat flavor soon lost its fishy characteristic. The fatty-soapy note perceived at a moderately strong level at first, slowly decreased in intensity but never disappeared. The serum, brothy, and salivating characteristics developed rather slowly. The mouthfilling factor slowly developed also, but bouquet barely was noted in the right loin

and not at all in the left. The metallic and astringent notes of the flavor which had developed 21 hours after slaughter were present in all steaks thereafter. After the test samples were swallowed, they left the mouth coated or greasy, and at no time was the aftertaste even remotely describable as satisfying. The panel volunteered that the 14-day steak was edible but not very pleasant.

Flavor Comparison of U. S. Choice and U. S. Commercial Grades of Sirloin

If one were to plot flavor with respect to time, the curve for U. S. Choice would begin at a higher flavor level than that for U. S. Commercial, and its curve would rise more steeply, ending at a considerably higher level of flavor. Plots for tenderness and texture would have similar curves. At no time during the period studied did the Commercial-grade beef match the Choice-grade beef in flavor or texture.

When hot, neither grade demonstrated identifying beef-steak characteristics. But the Commercial grade had corn-like and soapy characters completely inappropriate to the flavor of steak. As the Choice meat aged, it went through a stage where taste and feeling factors dominated any aromatic and bloom factors; then these last factors began to develop until the final flavor was a blend of sweet and serum with brothiness and animal, and with salivation, mouthfulness, bouquet, and a mouth-satisfying aftertaste.

As the Commercial-grade meat aged, it too went through a stage emphasizing taste factors and astringency, but its fatty-soapiness never disappeared, nor was the final flavor considered a good blend of notes. One of the most important factors lacking in the Commercial-grade steaks was bouquet—the release of a high burst of flavor each time a bite was chewed.

Comparison of Appearances of U. S. Choice and U. S. Commercial Grades of Sirloin

The hot Choice grade of beef was fairly soft and flabby. The lean was bright blood-red, while the fat was slightly yellow. The surface fat was only loosely attached to the lean, with a thin, tough fibrous membrane between the two. The fat was very rough and uneven, and so flabby as to be hard to cut. In the hot

Commercial-grade beef, the lean was a deep (but not dark) red and had a more moist appearance than the Choice beef. Also it was soft and greasy to the touch. The fat was a bright orange-yellow, very greasy to the touch, and rough and pebbly. As in the Choice-grade meat, the fat was not well attached to the lean and the fibrous membrane also was present.

When broiled, the hot Choice-grade meat was rather gray inside, like roast veal, and the fibers were very evident. The broiled Commercial steaks had a glossy, greasy surface, very dark in color. The inside was a blackish red, with very yellow fat deposits throughout.

The dish gravy from the broiled hot Choice steaks was watery and only slightly red; there was very little juice from the Commercial steaks.

After 24 hours without refrigeration, there were no changes in gross appearance of the Choice beef either before or after broiling. However, when the Commercial meat was broiled it resembled the better grade meat more closely than it did at the 3-hour panel.

After having been left on the animal in the chilling room for 24 hours, both grades of meat showed striking differences. In the Choice grade, the meat was firmer and kept its shape better, although it was still bright red. The fat had become a creamy white, much firmer and smoother, and adhered much more closely to the lean. The layer between the fat and lean was not as prominent as with the hot meats. The Commercial-grade meat showed the same general improvements; both lean and fat were much firmer, the fat was smoother, more closely attached to the lean, and lighter in color, although still yellow.

At the 48-hour period, the raw Choice meat was firm and a slightly deeper red in color. The fat had smoothed out considerably, was a consistent creamy white, adhered closely to the lean, and the fibrous layer had become almost unnoticeable.

At the same period, the Commercial beef showed comparable changes. The meat was firmer, less black-red when cooked, and slightly less greasy to the touch; while the fat was also firmer and smoother. However, this meat at no time looked like the higher grade meat, chiefly because of the very yellow fat and the greasiness in both appearance and to the touch. There were no further appreciable changes noted in appearance in either grade from the 48-hour panel throughout the remainder of the studies.

U. S. Choice Tenderloin

The panel also examined the two tenderloins taken from the animal described in tables 5 and 6 in order to provide additional flavor information. Table 9 summarizes the panel findings. The left tenderloin was entirely removed from the carcass 24 hours after slaughter and was taken to the Flavor Laboratory where it was loosely wrapped in aluminum foil and held at 38° F. Steaks 1 to 1½ inches were cut as required. The right tenderloin was allowed to age on the bone until the ninth day after slaughter. After its removal it also was wrapped in foil and held refrigerated until steaks were required.

Table 9. Effects of aging on broiled U. S. choice tenderloin (carcass weight—720 lb.)

	Left: Boned out at 24 hours		Right: Boned out at 234 hours —salted ad lib		
Age in hours	30	54	78	240	360
Steak No.	1 & 2	5 & 6	9 & 10	1	3
Aroma Notes and Their Intensities					
Amplitude	0	1–2	1–2	2	2
Brothy	0	)(	1	1–2	2
Animal	2–3	2	3	2–3	2
Nosefilling	0	0	)(	1	1
Sour	—	2	—	—	—
Fatty	2	1	—	—	—
Flavor Notes and Their Intensities					
Amplitude	)(	1–2	1–2	1	1–2
Sweet	)(	1	1	1	1
Serum	1–2	—	—	—	1
Brothy	0	0	1–2	1–2	1–2
Animal	4	3	1–2	2	2
Mouthfilling	1	—	1	—	1
Salivating	0	0	1	1	1
Bo iquet	0	+	+	—	+
Sour	2	1–2	1	1–2	2
Metallic (Met)	+	+	—	—	+
Astringent	+	+	—	+	—
Nosefilling	0	0	0	0	)(
Fatty Acid (F)	2	2	—	—	—
Aftertaste					
	Sour. Unpl.	Met. Sour. F	— —	Sour. Met.	Sour. Met.

Table 9 follows the setup of the tables pertaining to the sirloin steaks. Note that the panel members added salt to their samples before examining the last three steaks in the series. The character notes of the sirloin profiles were used, but it should be emphasized that the profiles of tenderloin and sirloin are different, though the differences are not as great as between beef and other kinds of meat. Two of the character notes require defining. Animal includes two aromatic factors: liver and kidney. Fatty refers to a fatty acid character.

The youngest samples were not recognizably tenderloin either in aroma or flavor. The predominating characteristic was the liver-kidney note, and this overrode even the fatty acid. Metallic taste and astringency were present, and the aftertaste was sour and unpleasant.

Twenty-four hours later a minimum of bouquet had developed and there was less sourness and animal character. The flavor notes were slightly more integrated. After another day there were additional gains in flavor, especially in mouthfilling characteristics. Such gains were not outstanding, however. The meat was very tender.

Steaks from the right loin, though aged longer on the bone, did not show the anticipated development of flavor. This finding correlates with those on the right and left sirloins from the same steer. Attention also is called to the fact that the total flavor at 15 days had not reached the amplitude levels of sirloin steaks of the same age. This finding explains the longer aging period accorded hotel cuts of tenderloin.

Summary

Using the profile method of flavor analysis an experienced laboratory flavor panel studied the development of beef flavor in broiled loin of beef steaks which had been aged, usually on the bone, under commercial conditions. Successive steaks were cut from the 13th rib toward the round, beginning at 3 hours after slaughter of the steer (hot meat), then on the first day (green meat), and through 2 weeks.

To prepare for the aging program, the panel studied and characterized the flavor of retail and wholesale cuts of U. S. Choice sirloin and similar retail cuts of high quality veal, pork, and lamb. Although they had several characteristics in common, each of these meats was found unique in flavor. All emitted an aromatic note described as "animal." Beef was the most interesting, satis-

fying, and most flavorful. The aroma of beef consists of the animal note plus brothiness and an almost indefinable characteristic that seems to fill the nose. This nosefilling quality is perceived in the flavor as are other definable aromatics such as brothiness and animal. Taste factors derive mainly from the juice, and feeling factors such as mouth satisfaction and salivation combine with the other characteristics to give a high amplitude of flavor. The whole flavor spectrum repeats itself each time the meat is chewed.

From the aging studies it was found that hot meat from Choice-grade loins had almost no recognizable "beef-flavor"; day-old green meat showed a slight development of beef-flavor; between 2 and 10 days after slaughter, a general pattern of beef-flavor development occurred. Sourness, metallic, and astringency declined. Sweet, serum, and brothy appeared and gradually increased. In the meanwhile, all the flavor characteristics became increasingly integrated, making a blend around the animal characteristic. Finally, the full profile was perceived each time the meat was chewed. However, the aftertaste development lagged. Usually, there was some sourness and a lacking in nosefilling and mouthfilling factors—and salivation might not have been noted. When the proper aftertaste had developed, steak flavor was achieved. Further aging would usually increase the amplitude of steak flavor.

Commercial-grade loin steaks, although showing a somewhat parallel pattern, did not achieve the full flavor of U. S. Choice cuts.

The flavor development pattern for U. S. Choice tenderloin was much the same as for Choice sirloin, but the rate seemed slower. In fact, even when salt was added, the flavor amplitude of 15-day tenderloin was lower than that of unsalted sirloin of the same age.

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The Source of Chicken Flavor

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Our investigations were in the nature of preliminary studies on the preparation of extracts which can be used in the study of

chicken flavor. The type of flavor described is that produced in a typical chicken broth. We have made no attempt to study the complex flavor characteristics of such flavors as are produced in browning reactions in roasting, boiling or frying.

Location of Flavor Source in Tissue

Studies were made on white leghorn hens between the ages of 1 and 2 years. All evaluations were made on broths prepared in a standard manner.

We were first concerned with finding out which parts of the chicken contributed to the typical chicken flavor. The birds were separated into the following parts: skin, leg muscle, breast muscle, bones scraped free of muscle, whole blood, plasma, blood cells, and abdominal fat. These were frozen, lyophilized, and stored in moisture tight containers at −5° F. until they could be evaluated.

According to Bouthilet's findings *(1)*, the typical chicken aroma is produced between pH values of 1 and 7, becoming more pronounced as the acidity is increased. Blood fractions were tested in both the normal slightly alkaline broths and in acid broths. Muscle tissue produces a slightly acid broth, usually between pH 6.0 and 6.5.

Table 1. Comparison of chicken fractions for odor- and flavor-producing characteristics

Fraction	Odor	Flavor
Skin	Slight	Slight
Whole blood, pH 2.8	0	—
Whole blood, pH 7.5	0	0
Plasma, pH 2.5	0	—
Plasma, pH 8.7	0	0
Blood cells, pH 2.6	0	—
Fat	0	0
Leg muscle	+++	+++
Breast muscle	++	++

As seen in table 1, the only fractions which produced a typical chicken aroma and flavor were those from muscle. Skin produced a very weak odor and flavor suggestive of chicken.

Extraction of Flavor Precursors

We next tried several extraction procedures in order to prepare a concentrate of the precursors of chicken flavor. Extractions with purified petroleum ether and with absolute alcohol were made

at or below minus 5° C., and water extractions at 0° C. in order to minimize tissue changes which might affect flavor.

Extraction of the lyophilized muscle with petroleum ether or ethyl alcohol did not affect the flavor of leg muscle. Neither of these extracts produced a characteristic chicken flavor. The concentrated extract produced by ice-water extraction of leg muscle gave, on cooking, a characteristic flavor. Very little flavor was obtained in the broth prepared from the residue of water extracted muscle (table 2).

Table 2. Comparison of odor- and flavor-producing characteristics of variously obtained and prepared chicken extracts

Fraction	Odor	Flavor
Leg muscle	+	+
Pet. ether ex'd muscle	−	+
Pet. ether extract	0	0
Ethyl alcohol ex'd muscle	−	+
Ethyl alcohol extract	0	0
Pet. ether ex'd bone	0	0
Pet. ether ex'd skin	weak	weak
1st 3 ice water extracts of muscle	+	+
2nd 3 ice water extracts of muscle	0	0
Water ex'd residue muscle	0	0

These results agree, in general, with those of other investigators who have studied chicken flavor. Pippen et al. found that chicken fat received a low score for flavor, and no significant differences in flavor score were obtained when the ratio of fat in two samples was 19 : 1. When freeze dried meat was extracted with petroleum ether, no difference was found in flavor between broths prepared from the extracted and unextracted samples, but there was a difference in aroma *(5)*. This difference in aroma has also been observed in our experiments. Bouthilet *(2)* also observed that chicken meat extracted with alcohol and isopentane or with acetone still retained the characteristic flavor, whereas the fat did not.

Pippen et al. also found that bones and skin received low scores for flavor, and adding these to the meat resulted in lower scores for flavor than obtained for broth prepared from meat alone. Crocker, in a study of beef broth, also found that bones, marrow and fat contributed very little to the beef flavor *(4)*. Pippen also found that soaking chicken meat in cold water decreased its ability to produce flavor. Flavor could be restored by concentrating the extract by lyophilization and adding this extract back to the meat. He also found that the loss in flavor could be partly restored by

addition of the ash from the chill water either as chlorides or carbonates. Part of this effect was accomplished by sodium chloride alone *(6)*.

Analysis of Flavor Fractions by Panel

In analyzing the various fractions examined as sources of chicken flavor, we have made use of the flavor profile method as used at the Arthur D. Little laboratory *(3)*. In this method, samples are evaluated for specific aroma and flavor notes which are given an intensity rating. Flavor characteristics are referred to a specific standard which has been selected by the panel members. This method is more descriptive than one which evaluates the broth as like or unlike chicken.

Table 3. Descriptive terms used by panel for chicken broth aroma

Component	Standard
Sulfide sulfur	Boiled egg.
Bready	French bread.
Meat broth	"Meat broth."
Burnt	?
Ammonia	Ammonia (in alkaline samples).

Table 4. Descriptive terms used by panel for chicken broth flavor

Component	Standard
Sour	Tartaric acid 0.06%.
Sweet	Sucrose 1.0%.
Salty	Sodium chloride 0.3%.
Sulfury	Glutathione 0.03%.
Oily	Salad oil (textural not related to fat).
Monosodium glutamate	Monosodium glutamate 0.5%.
Bready	French bread.
Nutty	Brazil nuts or filberts.
Burnt	?

Tables 3 and 4 show the descriptive terms used by our panel for the aroma and flavor notes in chicken broth.

Pippen has found that the sulfur in a distillate of chicken broth can be entirely accounted for by sulfide. The principal nitrogen component in broth distillate was found to be ammonia, which had little importance as a flavor constituent *(7)*. It is immediately detected by the panel when the sample is made alkaline.

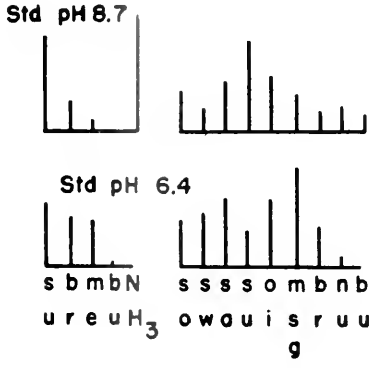

Figure 1. Comparison of chicken broths at standard pH and high pH.

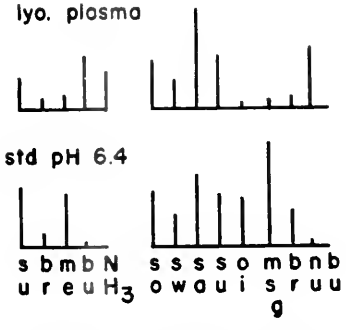

Figure 2. Comparison of high pH broth prepared from lyophilized plasma with standard broth.

In figure 1, a standard chicken broth at pH 6.4 is compared with a sample of the same broth that has been adjusted to pH 8.7. The most prominent flavor notes in the standard sample are those of *salty* and *monosodium glutamate*. In the alkaline sample, the sulfury note predominates in the flavor, ammonia appears in the aroma. In both samples the most characteristic aroma note is sulfide.

In figure 2, a broth prepared from lyophilized plasma, pH 8.7, is compared with a standard chicken broth. The flavor characteristics of plasma are quite different from those of either the standard broth or the standard broth adjusted to pH 8.7 (fig. 1).

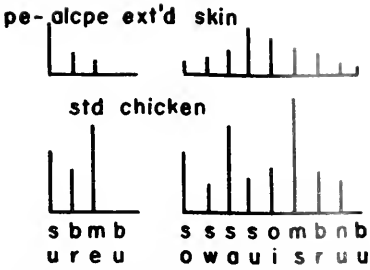

Figure 3. Comparison of broth prepared from solvent extracted skin with standard broth.

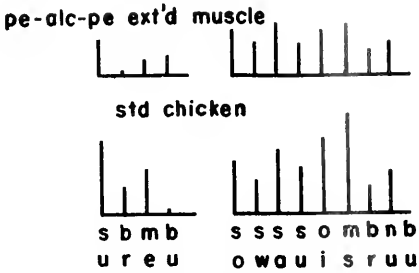

Figure 4. Broth made from solvent-extracted muscle compared with a standard broth.

In figure 3, broth prepared from solvent extracted skin is compared with standard chicken broth. In this comparison skin is quite different from muscle. This is possibly due to the fact that the solvent extraction removes components which are present in small amounts to begin with, since our earlier comparison showed that skin had a slight chicken flavor although extremely weak.

Figure 4 shows a comparison of broth made from solvent extracted muscle with a standard chicken broth. In this comparison the predominant flavor components are similar in muscle and whole chicken, but of lower intensity in the muscle. The aroma components are different in the fat-free muscle, the most notable difference being the weak sulfur component in the fat-free muscle.

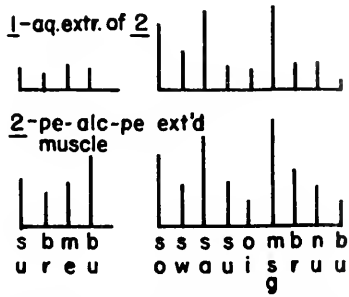

Figure 5. Comparison of broth prepared from fat-free muscle with broth prepared from aqueous extract of muscle.

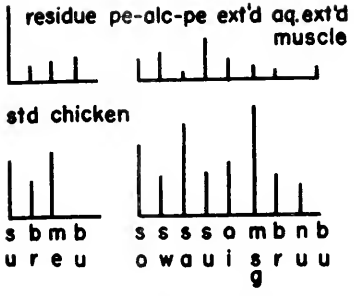

Figure 6. Standard broth compared with broth made from fat-free muscle residue after exhaustive solvent extraction.

Figure 5 shows a comparison of a broth prepared from fat-free muscle compared with a broth prepared from an aqueous extract of this muscle. The flavor picture is almost identical in these two, although the aroma is different.

Figure 6 shows a comparison of a standard chicken broth with a broth made from fat-free muscle residue after exhaustive extraction with petroleum ether, alcohol, and water. Very little flavor resides in the muscle residue. The principal aroma component is sulfide, which possibly arises from heat treatment of the protein.

Summary

A concentrate of chicken flavor precursors can be prepared by water extraction of lyophilized, lipid-free muscle. Fat itself appears to contribute little to the flavor of chicken broth, but in some manner contributes to its aroma.

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Chemistry of Coffee

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Coffee has been one of the most acceptable nonalcoholic beverages for many centuries and has attained its preeminence because it gives to the consumer a unique flavor experience. However, the chemistry of the stimulus, coffee flavor, underlying and related to this experience, has not been adequately developed. The acceptability of this beverage, like that of all other products enjoying wide distribution, depends upon a harmonious interaction of many factors which, taken together, comprise its history. A few of these factors are controlled by nature but, for the most part, both agriculture and industry are responsible for acceptability. How well they are able to take care of their obligation can be directly related to the extent of their experience. As is well known, experience can be developed through trial and error into an industrial art, or through research rigorously organized into an industrial science. In the case of coffee, agriculture and industry have fostered an art. Only recently, as the age of the commodity is reckoned, have they recognized the need for an organized scientific attack on the variety of unsolved problems with which they are confronted.

The purpose of this paper is to present certain pertinent facts so far as they exist, to show how much is unknown about coffee flavor and to point out deficiencies that can be eliminated only through research. The discussion will be arranged topically in accordance with limits that are logically established. These topics are: first, the composition of the green bean and factors that may alter it; second, changes in the bean during and after roasting; and third, the beverage itself.

The Green Bean

The consumer demand for coffee flavor is such that a huge mass of raw material must be imported each year. Approximately 2½ billion pounds of green coffee enter this country annually *(27)*; roughly 40 percent comes from Brazil, 25 percent from Colombia, 6 percent from Mexico, and 9 percent from all of Africa. El Salvador and Guatemala each contribute about 4 percent. The remainder, 12 percent, is imported from at least 20 other countries. The extent of variation among these coffees has never been

determined precisely, but whatever variability does exist must certainly be reflected eventually in the beverage. For reference, a few of the analyses that have been published are shown in table 1. These data are typical of those that have appeared in the litera-

Table 1. Composition of green coffee

	Santos percent	Mocha percent	El Salvador percent	Guatemala percent	Hawaii percent
Moisture	8.75	9.06	8.7	10.5	
Ether extract	12.96	14.0	8.2	5.0	18.2
Nitrogen			2.3	1.8	2.6
Protein	9.50	8.56			15.9
Crude fiber	20.70	22.46	21.3	23.1	13.8
Ash	4.41	4.2	3.6	3.2	3.6

ture—none of which permits the precise characterization of coffee with respect to area of origin. Occasionally measurements of other components have appeared, and from these a composite table (table 2) has been assembled to show, only crudely, what the green bean is.

Table 2. Composite analysis of green coffee

Component	Proportions, gm. percent	mg. percent
Water	8–12	
Oil (ether extract)	4–18	
Unsaponifiable	0–2	
Nitrogen	1.8–2.5	
Protein[1]	9–16	
Caffeine	0–2	
Chlorogenic acid	2–8	
Trigonelline	1–3	
Ash:	2.5–4.5	
Calcium		85–100
Phosphorus		130–165
Iron		3–10
Sodium		4
Manganese		1–45
Rubidium		traces
Copper		traces
Fluorine		traces
Tannin	2	
Caffetannic acid	8–9	
Caffeic acid	1	
Pentosans	5	
Starch	5–23	
Dextrin	0.85	

[1] Amino acids from protein: Alanine, aspartic acid, glutamic acid, glycine, leucine, phenylalanine, serine, thereonine, valine, cystine, methionine, and proline.

Table 2. Composite analysis of green coffee—Continued

Component	Proportions, gm. percent	mg. percent
Sucrose	5–10	
Reducing sugars	0–5	
Cellulose	10–20	
Hemicellulose	20	
Lignin	4	
Vitamins (present in small amounts): Carotene, thiamine, riboflavin, folic acid, niacin, pantothenic acid, citrovorum factor, B–6, and B–12. Ascorbic acid: none. Choline: 60 mg. %.		

There are also reports, as might be expected, that the composition of green coffees changes as the fruit develops on the trees, and later as it is processed by different techniques. Green coffee, if stored dry and away from disagreeable odors, will retain its flavor potential for 3 or more years *(18)*. However, the color may be affected within a year even in dry storage *(2)*. The moisture content must be kept below 10 percent to prevent flavor deterioration and unpalatability *(24)*.

The Roasted Bean

Because green coffee is devoid of aroma and a water infusion is bitter and unpalatable, it must be heated under carefully controlled conditions to bring about the changes in chemical composition that are so well recognized and appreciated. This process, called roasting, is actually a mild pyrolysis during which the components already mentioned are decomposed at rates in accordance with their relative stabilities toward the heat applied. During the process, the green coffee, at first slowly, then more rapidly, loses its greenness and takes on the familiar brown color. The decrease in greenness with increase in temperature (figure 1) has recently been measured *(15)* and can be used as a guide for controlling the process. As the temperature approaches 400° F., the beans begin to pop and expand to nearly twice their original volume. By this time much of the flavor has been developed. Finishing the roast now requires only a few minutes. The process is stopped abruptly by a short water quench and rapid air cooling at a precise temperature end point, varying within a range of 390° to 450° F., dependent upon knowledge the operator has acquired from previous experience. Given a batch of green beans

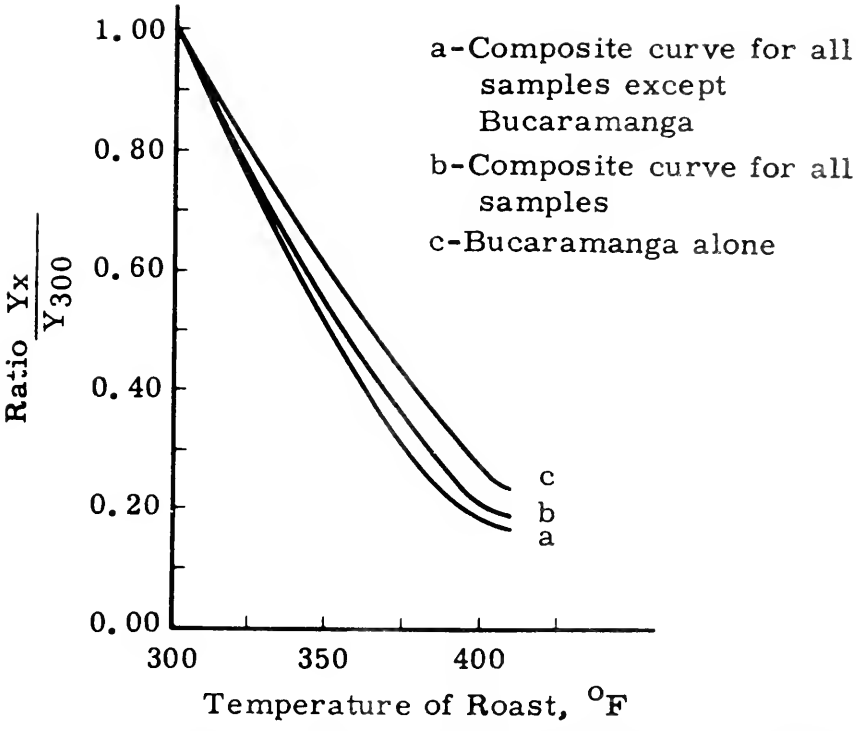

Figure 1. Decrease in greenness (Y-value) with increasing temperature.

of stated history, he knows that he must heat it to a specific brown color which is related to both rate of heating and final temperature, in order that the flavor characteristics most acceptable to consumers will be developed. By this very artful but indirect method, he is able to set up controls on flavor formation.

The question that is uppermost in the minds of all concerned, coffee processors and consumers alike, is what happens during the roasting cycle? Several general observations will be presented as an introduction to results of more specific analytical studies.

The moisture content of the beans is reduced to about 2 or 3 percent during roasting. There is a total loss in weight of about 16 percent *(20)*. This loss is made up of original moisture and of volatile decomposition products. Seven or 8 percent of the dry green coffee is decomposed and lost. The color of the beans changes to brown, and their volume increases from 50 to 100 percent. The acidity of aqueous solutions increases to a maximum and then decreases as roasting progresses *(19, 31)*; lightly roasted coffees have the highest acidity. Very obviously the aromatic properties change drastically.

From the proximate analyses that have appeared in the literature, it is very difficult to arrive at any clear-cut conclusions about what is happening. From the results shown in table 3 it appears

Table 3. Composition of raw and roasted coffee (dry)

	Protein percent	Fat percent	N-f extract percent	Sugar percent	Dextrin percent	[illegible] percent	Caffetannic acid percent	Ash percent
Mocha:								
Raw	10.8	13.8	.....	10.5	0.96	.....	9.3	4.1
Roasted	11.3	13.6	.....	0.4	1.24	.....	4.7	4.6
E. Indian:								
Raw	12.4	13.1	.....	9.9	0.93	.....	10.3	4.4
Roasted	13.3	13.6	.....	0.4	1.40	.....	4.6	4.9
Raw	12.9	13.2	42.4	8.5	0.96	27.8	10.2	3.4
Roasted	13.3	14.2	48.5	1.3	1.34	18.6	4.8	4.8
Kona:								
Raw	15.9	18.2	.....	7.8	5.8[1]	13.8	.....	3.6
Roasted	13.9	12.0	.....	1.9	6.8[1]	18.0	.....	3.1

[1] Starch.

that the protein and fat, except that from Kona coffee, are very stable. There is little change in the ash content. The greatest losses occur in the carbohydrate and caffetannic groups. Much of the decrease in weight is due to decomposition of sucrose and chlorogenic acid.

A more detailed analysis for specific components provides some understanding of the effects induced by heat. For example, in an extensive study of nitrogen partition in green and roasted coffee hydrolyzates, Underwood and Deatherage *(40)* show (table 4)

Table 4. Nitrogen content of coffee hydrolyzates

	Nitrogen per gram of dry bean		
	Total mg.	Basic mg.	Nonbasic mg.
Green Santos	21.8	10.7	11.0
Roasted Santos	19.8	9.6	9.9
Water extract of green Santos	12.5	7.0	5.5
Water extract of roasted Santos	5.7	4.8	0.94

that a slight decrease in total nitrogen is accounted for by relatively large decreases in the basic and nonbasic fractions of aqueous extracts. Their analysis of amino acid stability (table 5)

Table 5. Amino acid stability

	Nitrogen per gram of dry bean			
	Green bean mg.	Water extract mg.	Roasted bean mg.	Water extract mg.
Alanine	0.74	0.36	0.72	0.03
Aspartic acid	1.35	0.70	1.20	0.14
Glutamic acid	2.32	1.18	2.00	0.27
Glycine	1.23	0.59	1.09	0.18
Leucine	1.42	0.69	1.26	0.10
Phenylalanine	0.52	0.26	0.46	0.02
Serine	0.42	0.22	0.36	0
Threonine	0.27	0.14	0.22	0
Valine	0.62	0.28	0.56	0.03
Total	8.90	4.42	7.87	0.77

clearly indicates that some acids are much more stable than others and that those in a fraction extractable with water undergo considerable decomposition. The presence of sulfur-containing amino acids, cystine and methionine, in extracts of roasted coffee also has been reported *(3)*.

Tannins, including chlorogenic acid, are partially decomposed *(13)* in direct relation to the degree of roast. Approximately 50 percent of both chlorogenic acid and true tannin is lost in a heavy roast. These findings have been confirmed *(9, 12)*; p-vinyl guaiacol has been identified as a decomposition product *(30)*.

The decomposition of trigonelline has been reported *(10)* and confirmed *(23)*. The extent of decomposition again depends upon the degree of roast, but estimates of the loss have varied with the method employed. The most conservative figure for the loss is 15 percent. Data relating to the caffeine content of green and roasted coffee indicate that this component is quite stable. Losses that do occur are more likely related to sublimation than decomposition.

The evidence relating to the effect of roasting on coffee oil is conflicting. Several studies *(4, 5, 14, 26)* report no appreciable loss or change in components or in analytical constants. One study *(36)* states that although the values for the ether extract are almost independent of variety, they differ after roasting with variety and degree of roast. It is generally conceded that phosphatides, which are found in green beans in amounts ranging toward 2½ percent, are almost completely destroyed *(5, 35)*.

It is clear that a vast amount of work still remains to be done to enlarge upon and clarify the picture of green coffee decomposition during roasting. The effect of time and temperature of

roasting on trigonelline (fig. 2) by Hughes and Smith *(11)* is a fine example of what can and should be done for every component found in green coffee.

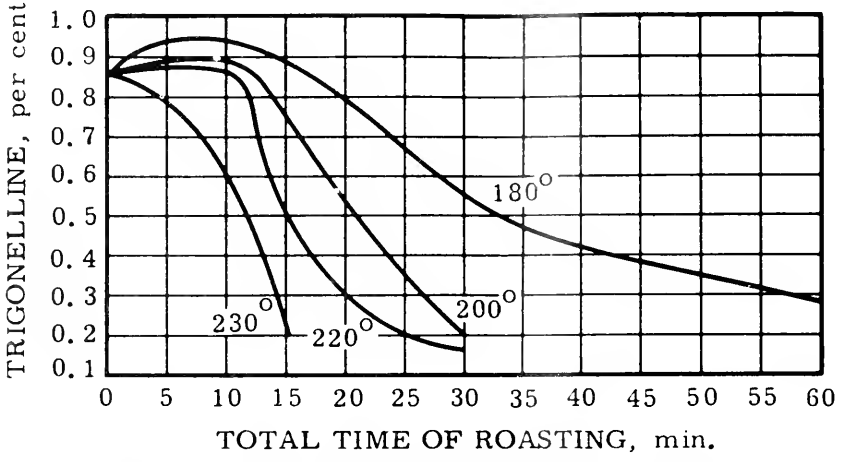

Figure 2. Decomposition of trigonelline during roasting.

Perhaps a knowledge of what happens to the components of green coffee during roasting is of somewhat less importance than a knowledge of chemical constituents retained by roasted coffee. In particular, those constituents that actually contribute to the taste and aroma of coffee beverage must be isolated and identified. Numerous studies on isolation and identification of constituents in roasted coffee have made their appearance during the past century. Little attention has been given to the quantitative aspects of constituent generation or retention and even less to concentrations available in terms of taste and olfactory stimulation.

Several good reviews on the constituents of roasted coffee have been written within the past 10 years *(11, 21, 22, 34)*. Extensive use is made of them in this one. Representatives of many different classes of organic materials have been identified. Some are simple aliphatic and aromatic compounds; others are complex and contain nitrogen and sulfur.

The major components, the unchanged protein, fat, carbohydrate, caffeine, trigonelline, chlorogenic acid, and tannin already have been mentioned. The term—"unchanged"—is used with understandable reservation. In all, 17 amino acids have been reported. Non-volatile acids such as acetic, pyruvic, caffeic, chlorogenic, oxalic, malic citric, and tartaric were identified and measured quantitatively *(17)*. In a continuation of this work in

which partition chromatography has been a very powerful analytical tool, a number of compounds that will undoubtedly join these are awaiting identification. Most of the vitamins escape complete destruction, while one of them, niacin, enjoys the unusual circumstance of increasing in concentration as the temperature of roasting increases *(39)*.

With few exceptions, all of these are non-volatile and were isolated from or measured directly in extracts. Chemists have been much more concerned with the volatile constituents in an effort to discover the dominant factors in coffee aroma. Some of them have worked with condensates of roaster gases with little success. However, this work was done more than 50 years ago. If an attack on roaster condensates were made today with techniques now available, there is no doubt that it would be significantly fruitful.

The best qualitative results have been obtained by dry vacuum distillation. The volatile distillate was collected in a series of traps cooled in stages down to the temperature of liquid air. Further purification gave a very unstable oil which on dilution smelled strongly of coffee. This concentrate was a complex mixture from which more than 70 pure compounds were isolated and identified. Even this impressive figure does not include all of the compounds present. The search still goes on. A partial list of constituents sufficiently volatile to be identified in condensates and distillates is given in table 6.

Table 6. Volatile components in coffee aroma

Formic acid	Phenol
Acetic acid	Resorcinol
Methyl ethyl acetic acid	Cresols
M-Valeric acid	Ammonia
Iso-valeric acids	Methyl amine
Higher fatty acids	Trimethylamine
Ethyl alcohol	Pyrrole
Acetyl methyl carbinol	N-methyl pyrrole
Furfuryl alcohol	Pyridine and homologues
Acetaldehyde	Pyrazine
Methyl ethyl acetaldehyde	Guaiacol
Furfural	p-Vinyl guaiacol
Acetone	Eugenol
Diethyl ketone	Hydrogen sulfide
Diacetyl	Methyl mercaptan
Acetyl propionyl	Dimethyl sulfide
Hydroquinone	Furfuryl mercaptan
Esters	Furane
Furfuryl formate	Sylvestrine
Furfuryl acetate	Vanillone
Methyl alcohol	n-Heptacosane
2,3-Dioxyacetophenone	

A most remarkable situation has developed from this combined extensive examination of coffee aroma. There is no single chemical component that can be held responsible for what every coffee drinker recognizes as coffee aroma. One of the striking facts that differentiates the coffee complex from that developed in roasted chicory, wheat, rye, barley or other plant materials that have been used unsuccessfully as substitutes for coffee is the presence of sulfur compounds in coffee. A great deal of enthusiasm arose when the mercaptans, furfuryl mercaptan in particular, were identified. Although mercaptans as a class are considered to be extremely offensive, at dilutions of a few parts per million or billion they take on an entirely different character. When furfuryl mercaptan is so diluted, it is generally conceded to have an odor reminiscent of coffee. However, under no known conditions that have been devised to date can it be made to serve by itself as coffee aroma.

In another experiment *(22)*, an oily distillate from coffee was treated to remove pyridine. The resultant complex did not have a true coffee aroma. When pyridine was added back to the complex an aroma more nearly like coffee was produced. This experiment, so far as is known, has not been repeated nor has the result been confirmed. However, it brings out an important aspect of flavor analysis. It is almost impossible to predict what the addition of one aromatic to another or to a complex will yield. Blending, masking, and enhancement of odors and tastes are still mysterious operations. Information about them generally is obtained by trial-and-error and through experience.

Two other components that may play an effective role in coffee aroma are diacetyl and acetyl methyl carbinol. Although the odor of neither of these compounds resembles that of coffee, they are present in sufficient quantities to add notes to the complex. Their true odor may be lost in the complex but nevertheless contribute to it.

Many of the other constituents such as the simple ketones, acids, aldehydes, alcohols, and esters are relatively poor odorants. This statement is made on an intuitive basis because the literature contains practically nothing on olfactory thresholds. However, it is quite possible that the additive effects of small quantities of these constituents may be sufficient to produce an effect. The measurement of olfactory thresholds and of changes in olfactory effect resulting from the mixture of 2 or more chemical compounds makes up an area of study which, fundamentally, would provide information useful to all food flavorists. It is of great importance to the coffee industry because of an apparent com-

plete absence of a single dominant aromatic component or even 2 or 3 components that can produce a desi: ble or acceptable sensation. Studies of this kind would be extremely useful to manufacturers of regular coffee for brewing, because they would lead to an efficient saving and protection of flavor produced and initially present in roasted coffee. Work in this field might solve some of the problems facing manufacturers of soluble coffee, because only by reintroduction of the aroma lost during processing can a truly quality product be presented to the consumer.

Although much effort has been expended in identifying the aromatics present in roasted coffee, little has been done to find out at what rates they develop in or disappear from roasted coffee. The only results of basic value that have been published come from work of Hughes and Smith *(11)*. They have shown (fig. 3) that

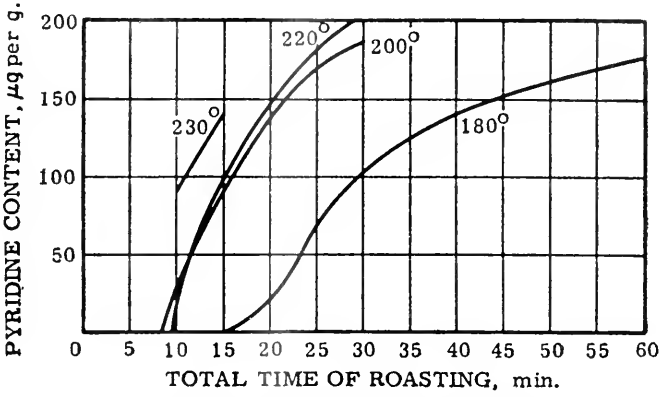

Figure 3. Increase in pyridine content during roasting.

for times and temperatures associated with normal processing the pyridine content of coffee will increase. The times are not long enough nor the temperatures high enough to produce a maximum followed by a decline. Formation of pyridine parallels trigonelline decomposition. In further work in which, unfortunately, analytical results were related to percentage weight loss of roasted coffee, the furfural content was shown to be already at a maximum when the coffee weight was reduced by 12 percent. From this maximum, there was a rapid reduction as the roasting loss reached 18 percent. Aldehydes are produced only slowly as roasting loss increases to 12 percent, but thereafter the rate accelerates, with no indication that it will reach a maximum and decrease within the normal range of roasting conditions. Diacetyl and acetyl methyl carbinol were found in approximately equal amounts and, excluding over-roasted coffee, the total amounts

were independent of the extent of roasting. Since it can be inferred that both of these compounds are continually being formed during roasting, they must be undergoing further transformation at approximately the same rate. The acetone content increased as roasting proceeded. Here again there was no evidence that a maximum had been reached and that the acetone content would decrease either through volatilization or decomposition. The same was found for volatile phenols; there was an accelerated rate of formation with no evidence of a maximum value within the normal roasting range.

These results, which were reported in 1949, represent a major contribution to the pool of coffee information. They stand out as a dramatic emphasis of how little has been done and how much there is to do.

A new attack on the chemistry of coffee aroma recently has been initiated. The work was started only because a new analytical tool, ideally applicable to all types of problems involving micro-concentrations of odorants, has made its appearance. Vapor chromatography, or fractometry, is now recognized as a technique that will open the doors of many flavor problems. Simple in principle and in use, it will permit concentration of mixtures, such as coffee aroma, both qualitative and quantitative separation, and identification of individual components, and finally concentration of separated fractions. Only a few of the preliminary results need be presented at this time to show the potential of vapor fractometry. In figure 4 will be seen a typical fractionation

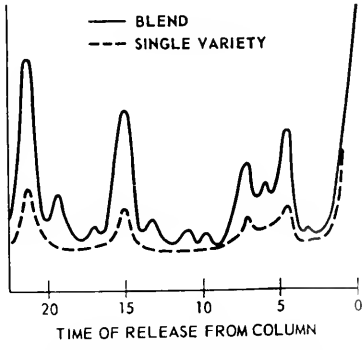

Figure 4. Vapor phase chromatography of coffee (blend vs. single variety).

of very small samples pulled by vacuum from two coffees, the one a single variety and the other a blend. It is certain from what was said previously about the number of compounds already identified in coffee that the whole picture of the aroma is

not visible. However, it is apparent that these two aromas differ not only in complexity but also in quantitative aspects. With refinement there is no doubt but that this technique could be used to assist in the blending of green coffees for development of new flavors, for the control of roasting, for the attainment of constant flavor, and for the measurement of the progress of staling in relation to packaging and shelf-life. A first look at freshness through the eyes of the fractometer is presented in figure 5. Here are the

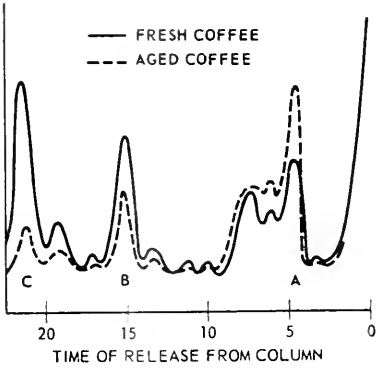

Figure 5. Vapor phase chromatography of coffee (fresh vs. aged coffee).

analyses of the aroma from two samples of the same coffee taken 66 hours apart. Although the qualitative features of the fractionation have not changed significantly, there are differences in concentrations. Note the general decrease in the heights of peaks at B and C, indicating loss probably because of volatility but, in particular, the increase at A in one of the more volatile components. Finally, figure 6 shows progress toward identification. The fractometric characteristics of acetaldehyde and acetone agree well with those of two components of the coffee complex. Because identification of components is most easily accomplished if knowledge of what may be present is at hand, the patient and painstaking efforts of the many chemists who already have made important contributions will make future progress with the fractometer rapid and productive.

Gone are the days when green beans were roasted in the home, ground, and used within a short time after preparation. Now the time interval between preparation and use is more likely to be weeks or months. The roasted beans, whether whole or ground, packaged in paper bags or in vacuum cans, undergo changes that lead to deterioration and unacceptability. Attempts to understand and prevent these changes have led to packaging in cans

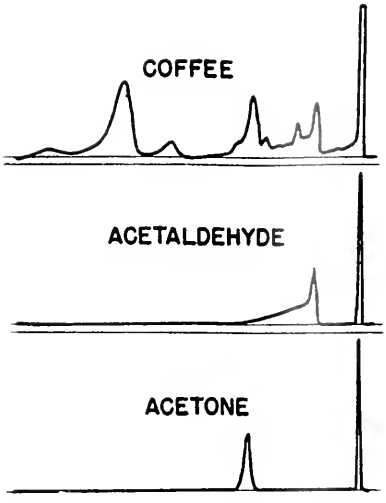

Figure 6. Comparison of fractometric characteristics of acetaldehyde and acetone with those of 2 coffee complex components.

under vacuum or under pressure. The search for better flexible packaging materials still continues.

There are several aspects to this problem. The scientists who have studied it seem to agree that the main factors are loss of freshness, staling, and rancidification. Freshness is said *(33)* to involve a volatilization and sweeping out of aromatic components by gases formed during roasting. These have been estimated *(32)* to consist of carbon dioxide, 90–95 percent, and carbon monoxide, 5–10 percent. Whether the carbon dioxide is held in the cells of roasted coffee under high pressures, reported *(32)* to range from 110 to 170 pounds per square inch, or held in chemical combination and released by the action of moisture as has been suggested *(6)*, is beside the point at the moment. The fact is that carbon dioxide and other gases are given up slowly by roasted whole coffee, more rapidly by ground coffee, and extremely rapidly if the ground material comes in contact with water. Volatilization must occur, because coffee exposed to the air gives off a powerful aroma. The effect of mechanical sweeping by carbon dioxide is doubtful. In fact, even sweeping freshly ground coffee with dry nitrogen for long periods of time did not affect the aroma appreciably *(38)*. The only direct measurements that have been made on aged coffee *(11)* indicate that no loss of pyridine occurred within 7 months, no loss of furfural within 6 weeks, a slight loss of aldehydes, acetone and phenols within 6 weeks, a moderate loss of diacetyl and acetyl methyl carbinol within 2 months, a conversion of diacetyl to acetyl methyl carbinol and a

rapid loss of hydrogen sulfide. A decrease in freshness is probably due to volatilization.

Staleness seems to be caused by oxidation of aroma components with the development of nonaromatic oxidation products. This occurs very slowly at first because of a protective action of carbon dioxide. As the carbon dioxide content of the roasted coffee decreases, there is an increasing opportunity for oxygen to attack the aromatics. Noticeable staleness is detected after about 10 days and is quite apparent after 3 weeks. Prescott and his coworkers *(29)* found no evidence that would associate decomposition of coffee oil with staling.

Rancidity, a flavor phenomenon resulting from an interaction between coffee oil and oxygen, develops extremely slowly. The stability of coffee oil is increased by roasting *(8)*. This probably is due to the formation of heterocyclic imino compounds, some of which have been shown to be anti-oxidants. The oxygen absorption induction period of the oil was found *(7)* to be a reliable measure of its tendency to go rancid, and is substantially constant for 13 weeks after roasting, whether coffee is stored in air or in vacuum. Since coffee stored in air becomes stale within 2 weeks, staleness cannot be synonymous with rancidity. True rancidity may not be apparent until after 7 or 8 months *(32)*. Vacuum packed coffee seems to be stable indefinitely.

Although a picture of what happens to coffee after it has been roasted can be presented, it still is based largely on inference from the few facts that have been published. Nothing is known about the relative stability of different types of coffee or about changes that may occur in flexible packaging materials now becoming available. The whole subject of roasted coffee stability needs reinvestigation and warrants extensive research.

Coffee Beverage

Just as green coffee is raw material for the roasting process, roasted coffee is raw material for beverage preparation. The making of beverage is a simple extraction procedure, but it requires just as much strict adherence to formulation and technique as does the preparation of any other food for table use. Because coffee beverage is acceptable over a wider range of quality variation, less attention has been given to its preparation by the consumer than to that of other foods. Manufacturers also have expended less effort in developing information about the end use of their product

than about the product itself. Fundamental facts about the beverage are only rarely encountered in the literature and usually are totally unrelated to common preparative practice.

The factors involved in beverage preparation are these: particle size distribution of ground coffee, ratio of coffee to water, water composition, water temperature, time of contact between grounds and water, metal contamination, and type of equipment.

Roasted coffee beans are ground to permit rapid absorption of water and rapid extraction of the soluble components that comprise flavor. Particle size distribution has been studied with the result that certain arbitrary limits have been established for grinds popularly called regular drip, and fine. An analytical description of these grinds has been published *(41)*, but at the present time it serves only as a manufacturing guide and a basis for military specification. There is no information concerning the uniformity of grinds designated by the terms mentioned nor by other terms in common use such as extra fine, all-purpose, urn, or Silex. However, because absorption of water and extraction take place at a rate which is inversely proportional to particle size, a greater knowledge of grinds must be acquired in order that the mechanical and physical features of brewing equipment be utilized to best advantage. Ideally, particle size should be held within very narrow limits and custom fitted to equipment specifically designed to provide optimum extraction.

Almost all of the information available on the brewing process and the factors involved is contained in the Prescott report *(28)*. He and his coworkers concluded that very hard or very alkaline waters exert an unfavorable influence on the character of beverage coffee. Ordinary soft waters or waters of low hardness yield the best beverage. Others *(16)* have shown that the concentrations of impurities in most municipal water supplies are not great enough to be perceptible in the beverage. However, they may be great enough to affect quality by altering the extraction process. Prescott states that the time of infusion, that is, the time of contact between the grounds and water, should be brief. Work now in progress shows that the time of infusion may be affected markedly by the chemical composition of water. This is shown in figure 7. Synthetic waters of simple but known composition percolate through a standard bed of coffee at different rates. Any alteration of the chemical composition that increases the concentration of sodium carbonate or bicarbonate tends to increase the percolation or infusion time. Zeolite softener treatment of hard water containing a high bicarbonate content will cause an increase in percolation times. The coffee grounds themselves act in a

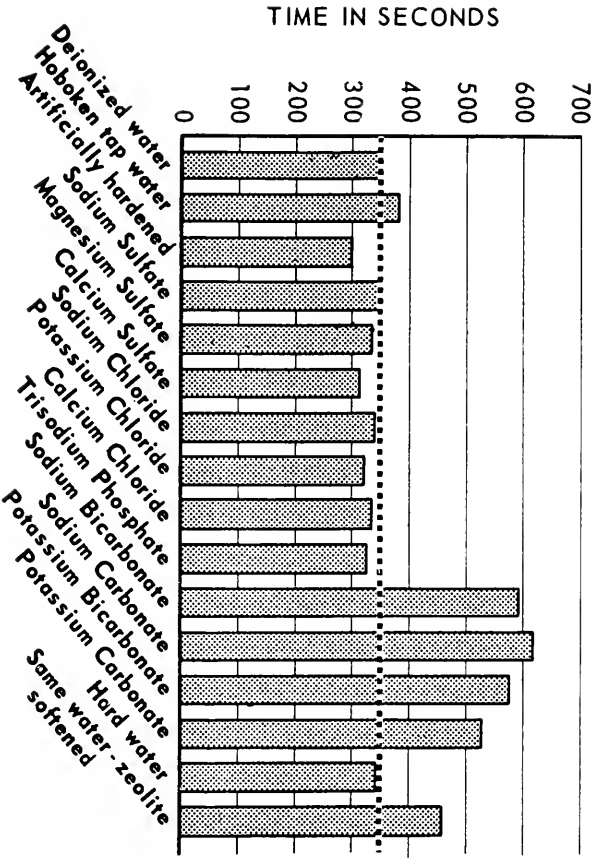

Figure 7. Percolation times for waters containing different minerals through coffee.

minor way as a softening agent by reducing the calcium content of brewing water *(25)*. Because long infusion or contact time increases the bitter taste and decreases the flavor and aroma *(28)* any water treatment that tends to increase this time should be avoided. Cup testing *(1)* also has demonstrated that coffee made with untreated water was preferred to that made with treated water; that made with hard water was preferred to that made with distilled water, but the latter made better coffee than water passed through a softener.

The temperature of the water used should be very hot, at least within a range between 185° and 203° F. At these temperatures, the caffeine is almost all dissolved, the aromatic components are not boiled off, and changes resulting in bitterness and woody taste are absent or negligible. Coffee should never be boiled, because

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such coffee is markedly more bitter than that prepared at 203° F. *(28)*.

The action of beverage on metals is pronounced and bitter, astringent, or metallic tastes may be produced. Tinplate, aluminum, copper, and nickel are listed in order of decreasing adverse effect. Glass, porcelain, and other similar materials have no noticeable effect on coffee flavor *(28)*. Stainless steel is resistant to attack, so its effect on flavor is probably negligible. As only trace amounts of the other metals are dissolved *(37)*, their action is undoubtedly catalytic.

The composition of beverage coffee is intimately related to the weight-volume ratio of the dry coffee and water used in preparation. Although some variation in this ratio does exist, expert coffee tasters generally agree that a 6 percent weight-volume ratio will yield an excellent product, if all other factors are rigidly controlled. Such a product will have a solids content of approximately one to 1½ percent. It will contain as major components caffeine, trigonelline, chlorogenic acid, and organic acids such as caffeic, malic, citric, tartaric, oxalic, pyruvic, and acetic. Tannin and amino acids are also present in small quantities. Aromatics such as furfural, simple aldehydes, phenols, and hydrogen sulfide, in beverage have been identified, and the quantities measured decrease as the beverage stands *(11)*. This is the first instance of an attempt to find out why the flavor of beverage changes on standing. However, a more complete quantitative examination of component distribution and stability in the beverage comprise problems that currently are being investigated.

In summary, then, a great deal of work has been done on coffee flavor. However, the number of factors involved is so great and the interrelationships are so complex that the proportion of effort expended on any one phase of the subject is relatively small. Research now in progress will undoubtedly expand considerably the knowledge about coffee flavor and assist in solving many existing problems.

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V. A SUMMING UP

Where We Stand Today in Flavor Research

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Each paper given in this symposium is, in effect, a summary of an important field of present day flavor research, and it is not my intention to give a summary of summaries, but rather to pass on to you some thoughts which have occurred to me while working with essential oils and flavors.

Some might think that I am an ex-expert in the field of flavor chemistry. This is not quite true, for I am engaged at the present time on a super flavor problem—the flavor of Los Angeles. It is remarkable how closely this problem does tie in with flavor research. The methods in both fields are nearly identical.

The basic reason why we have air pollution discomforts and the purpose of our gathering here to discuss the flavor of our foods are very much the same. In both cases, it is the greater complexity of our living conditions and a rapidly growing density of populations which create problems unknown to our forefathers.

For Critical Consumers—Condiments

In the transition time when mass feeding became necessary, many mistakes were made unknowingly, and large scale cases of malnutrition have occurred. The classical example of this was the occurrence of vitamin B deficiencies in feeding whole populations with polished rice, and the appearance of scurvy through vitamin C deficiencies on ships. With the knowledge that a certain amount of calories and specific food substances are essential for survival, cities and armies now are being fed without apparent ill effects with a diet in which the components have undergone quite some changes before they reach the consumer. This does not mean that we have reached ideal conditions in large scale processing and feeding. The food produced in this manner usually has lost some of its original flavor, and the development of off–fla-

vor is recognized instinctively as coming from inferior food. There is a good, scientific basis for this instinctive feeling. Both flavor substances and the essential vitamins are delicate structures which undergo changes readily through heating and the action of chemical or biological agents. With the present skill of the flavor chemist, it is possible to cover up these deficiencies to a certain degree. The poor consumer meanwhile has been driven to the use of tremendous quantities of extraneous flavoring materials, and has become addicted to the use of catsup and barbeque sauce in making his flavor-poor diet more attractive. Since the individual no longer is able to use his old criteria of wholesomeness, he is practically at the mercy of the food processors.

While, on the whole, responsible elements in the food industry will not misuse their ability to disguise inferior materials with first-quality flavor, there is no doubt that people need to be protected by more and more stringent food and drug laws as the ability to modify our food flavors increases. However, in most cases, we are not yet that far. Everyone will agree that the flavor of most artificial and concentrated foods can stand a tremendous amount of improvement. While I was writing these few notes I had in front of me a cup of bouillon made from hot water and a cube which was supposed to have all the ingredients commonly associated with chicken broth. The actual taste was that of salty water, with an odor reminding me of my freshman organic laboratory course when we boiled hair with hydrochloric acid for the preparation of cysteine. This is only one example out of many where it is obvious that a great deal of work has still to be done on a true reproduction of the natural flavor.

There is, of course, no doubt that with the increasing density and urbanization of the population, mass production of food will increase. With mass production came problems of keeping the food as much as possible in its original condition. For this reason, enzymatic action as well as bacterial and other microbiological activity must be stopped, and sterilization methods become practically unavoidable to preserve food for the longer period of time necessary to bring the product from producer to consumer.

Confronting Flavor Complexities

The changes induced in these processes are highly complex. Anyone knows, for example, how boiling destroys the delicate flavor of milk and replaces it with a peculiar new flavor, which we indicate as "cooked flavor," for lack of a better description. Con-

tinued research has produced in the quick heat sterilization of milk a product which is acceptable to almost everyone, and which by some is even preferred to fresh milk. However, pasteurized milk has only a short shelf life, and as soon as we want to keep milk for a longer time off-flavors again are produced. In these processes numerous reactions have taken place. The formation of a small amount of hydrogen sulfide reveals a breakdown of proteins; brown coloration indicates a reaction between amino acids and sugars. Flavor components have disappeared, and new ones have been formed from originally nonflavor compounds.

While research on the original flavors and the transformation by physical factors such as heat already is extremely difficult, one can well imagine how complex the problem becomes when cheese- and butter-makers are through with the milk. I sympathize with those who work in the field of secondary and even more highly derived flavors, and would like to compliment these investigators on their courageous undertaking of such complex problems. Not only do we deal with the composition of the primary flavor of the material as it is still produced by the cow, but the product undergoes changes in the processing which involve the original flavor compounds as well as all cell components, and the final product will become extremely complicated in its composition.

A similar situation exists when secondary flavors of bread are being investigated. Here, even more complexities arise, for we have to deal with a mixture of vegetable and animal products, partly fermented and pyrolyzed at different temperatures to add the typical crust flavor to the rest of the bread. Since a flavor study of each one of the products which go into bread already would constitute a major task, we have to look with great respect at the amounts of information already assembled by our bread chemists.

Off-flavors and Smog

Lately, a new complexity has arisen by the use of radiation in the sterilization processes. While a desirable addition to our weapons for mass feeding, certain drawbacks have arisen in some instances where severe off-flavors result from the radiation effects. It is suspected that during the irradiation free radicals are formed, which in turn could form peroxy radicals, as well as hydrogen peroxide. There might be an interesting analogy with the photochemical events in smog formation. As you know, the smog condition in Los Angeles is largely caused by a photochemi-

cal peroxidation of organic material with oxygen of the air. The oxides of nitrogen function as light energy absorbers in this reaction. Here, too, we have adopted a working hypothesis that free radicals are formed, and these in turn give peroxy radicals. However, instead of forming hydrogen peroxide, as in the case of our food, which is mainly in aqueous medium, the reaction in air, where there is a large excess of oxygen, results in the formation of considerable quantities of ozone. In both cases, the substrates undergo the action of strong oxidizing agents. In foods, one of the first results is a damage to the flavor complex. In air, the first effects of the photochemical reaction are the increased cracking of rubber and an eye irritation in human beings.

It is, perhaps, interesting to the flavor chemist to know that a regular food component, biacetyl, with a sweet, butter-like odor, upon irradiation with sunlight forms ozone in the presence of oxygen, also through a peroxide radical mechanism. It is clear that flavor changes must result from the transformation of such a strong flavor compound. Moreover, the situation becomes much worse, because in the course of its decomposition biacetyl leaves behind strong oxidizing substances such as peroxides which can greatly accelerate further autoxidation.

Notwithstanding all the work which goes on at the present time to prevent the deterioration of the flavor of our foods in processing, it is not very likely that we can eliminate completely the bad effects, and our best hope is to minimize the worst features of some of the sterilization processes.

Men, Methods, and Machines

Fortunately, there are a number of chemists who are interested in determining the composition of the different flavors, and in this symposium we have heard many interesting contributions to our chemical knowledge of natural flavors and to the methods used in arriving at this knowledge. We have heard from Dr. Eskew how to get flavor components in bulk. Notwithstanding the ability of Dr. Stahl and Dr. Hasselstrom to cope with the identification of small quantities of material, I am sure that they will agree with me that there are times when one would like to have a bucket full of the right stuff.

When we see the development of all new methods—spectrographic, chromatographic, X-ray, and other means—sometimes think how much time we would have saved by simply waiting until the methods had been perfected before tackling our problem.

This is somewhat like saying that if Columbus had had some patience, America could have been discovered more conveniently by English warships. In both cases, we forget that the experience gained in the discoveries, with all their hardships, was a prerequisite to our present success. The laborious method eventually led to the ingenious devices which now are able to open many of the fields which were rather hopeless only a dozen years ago.

The Biochemical Studies

We have seen in this symposium interesting reports on the application of the newer methods to the analysis of cheese, bread, poultry, coffee, cabbage, citrus, and strawberries, all of which constitute a definite extension of our knowledge of flavor components. While I do not plan to go into a detailed discussion of these flavors, I would like to comment briefly on the general nature of the compounds formed. It is often found that we can divide the individual flavor components into groups of related biochemical origin.

In the study of pineapple flavor, we found that most of the constituents could have been formed from a reductive deamination of amino acids. The most interesting product found was a thiol propionic ester, which could be related directly to methionine or cysteine. It is of interest that the pineapple fruit carries with it a protein-splitting ferment, bromelin, and its activator, glutathione.

In other cases, we see a direct interrelation through fat or carbohydrate metabolism, and it is not difficult, as Drs. Hasselstrom and Hewitt have pointed out, to list the flavor components of other fruits and products according to their biochemical origin. Dr. Hewitt's experiments on the improvement of flavor through the action of enzymes support the general idea that flavor precursors occur, which are transformed later into the actual flavors. It is a most useful and interesting study for the flavor industry to find out exactly how these relationships come about. For this we have to know more about the basic mechanism of synthesis, and also degradation of natural cell compounds. Much progress has been made in this field by using isotope techniques, and biogenetic methods with neurospora have shown the complicated ways in which nature circumvents energy barriers in its syntheses. A great deal more work needs to be done before we are able to carry out these reactions *in vitro*. I am thinking especially of the synthesis of such well known flavor components as the terpenes.

Their origin is supposed to be the same as the fatty acids. But in the beginning of their synthesis, the acetyl or coenzyme A chains condense in a slightly different way than in the case of fatty acid synthesis. Instead of a straight chain, the typical regular branching appears, characteristic for all terpenes. It is tempting to find the proper conditions, the right enzymes, to effect this synthesis. We don't know exactly how this branching comes about, but it would be interesting and useful to know more about it, and to be able at will to change the course of fatty acid synthesis to give terpenes, and vice versa. We would need to know how to make those first addition products, and to find the enzymes which make the interesting diversity of terpenes. In this way, the knowledge of the biochemical processes will aid the flavor chemist in synthesizing many of the compounds which are available to him only through a long drawn out and costly synthesis.

Another field closely related to the biochemical studies which hardly has been touched is that of flavor development and intensification during the growth of the plant. Some investigations of this sort were conducted, but they never received the care which they deserved. In only a few instances have the optimum conditions for flavor development, such as temperature, length of day and night, and humidity been studied under properly controlled conditions. We should realize that the flavor story begins when the seed germinates, and, when we want to draw genetics into it, even before that time. Tremendous differences in flavor can be developed both by genetic means and by physiological control, as Dr. Went has shown at our Institute in studies on strawberries.

The Role of the Senses

Then there is another interesting problem that we must solve before being able to control more completely the human reaction towards odors and tastes. With a better understanding of the basic mechanism by which we experience these sensations, we ultimately might be able to replace the empirical method of producing valuable odor compounds by a more scientific method. It is always most surprising to me that our chemical senses are able to analyze so many compounds in only a fraction of a second. The nose, especially, is expert at this, and easily distinguishes between fatty acid homologues differing by only one carbon atom. The quaternary carbon atom carrying several methyl groups, mainly responsible for the typical odor of camphor, is recognized quite readily in a number of other compounds. More limited in

its powers of distinction, our sense of taste also catalogues a large number of molecular structures remarkably fast and accurately, according to a few basic impressions. When a solution tastes acid and a pH meter tells you differently, it is a sure bet that the instrument needs some adjustment, and not your tongue. Anyone working with flavors and odors has experienced the difficulty in describing one's impressions, and this lack of an adequate descriptive language has been bothersome in discussing practical problems and has hindered research in understanding basic mechanisms considerably. It looks now, however, as though we must first understand why we smell and taste in order that we may be able to properly catalogue our compounds according to their impression on our senses.

Penetrating the Language Barrier

In the past we have seen schemes which tried to fit the various odor impressions into a small number of classes. Each odor then could be described as consisting of a number of sensations caused by the simultaneous presence of different quantities of the basic odors. Such schemes have been developed in the past by Zwaardemaker, Henning, Crocker, and Henderson, and this interesting problem was discussed today by Dr. Pilgrim. Other speakers have added more difficulties to our language barrier, and I hope to see some day a scientific explanation of Dr. Caul's "mouthfulness." Such expressions, and many more used in this symposium, clearly indicate the need for serious studies in this direction. While there are evidently difficulties in obtaining significant correlations in flavor estimation, we should be glad that there are still courageous people who try to make us speak the same language, at least in food technology.

Flavor Sleuths—Human and Mechanical

Before closing, I would like to talk for a moment about the financial end of flavor research. Present day research in flavor is expensive. The modern apparatus without which we cannot adequately study the intricate problems come in multiples of ten thousand dollars. Often it is advisable to "purchase" at the same time someone who can operate the machines and get the most out of them. With these new instruments, the impression might be created that now all our problems are over, and that the only

thing to do is to funnel the product into the machine, and out comes a little note with the composition of the material nicely typed out. I don't have to tell you that this, unfortunately, is not so. Much ingenuity and technical skill are necessary to use the machine to advantage. We shall go through a period where almost everyone will cash in on the usefulness of these instruments. We shall in that way obtain a more complete picture of the materials we are working with, and we shall have a much more satisfactory picture of the known substances in flavors.

It is also true that there still are a large number of flavor compounds which have never been described, where we run up against unknown spectra and unidentifiable fractions in chromatographic procedures. It is then that we have to rely once again on the skill and persistence of the old flavor sleuth, with his tedious methods of collecting material and identifying the structure of unknown species of flavor molecules.

It is obvious that if research were left to the individual food grower or producer we could hardly expect a satisfactory coverage of the field. It is fortunate that these individual interests have been pooled in research institutes, and that Government agencies such as the Regional Laboratories, Army, and Navy all are contributing their share to flavor research.

Discussion

PATTON:

This is not directed to the speakers, but then I am sure that some others will join me when I actually say that I am reluctant to see this matter end here. I have listened to these very stimulating papers and I know that the audience has been most attentive. I see a big future for flavor research in the years to come and so I would like to know if we can do anything to make our group a more permanent kind of thing. I wonder if we can do anything about making our publication more effective so that we will not have to page through many other publications that are perhaps not relevant to our field and have all of our information concentrated in one place.

HAAGEN-SMIT:

I think we have a somewhat similar situation in the air pollution field. Until a year or two ago, all of the papers were stretched over all the journals and then it was finally decided that we would put our papers together. Of course, we certainly

can publish the papers that have been given here and they will be published in *Food Technology*. Therefore, I think that my advice would be that for the present these papers should be put in *Food Technology*.

DR. S. D. BAILEY (QM Research and Engineering Center):

With our modern instruments now turned in the direction of flavor isolation and identification, much new data is now available for basic studies on flavor origin and control. There is always the danger with new instrumental advances to concentrate on grinding out more results rather than to apply these results to basic processes. We should not let our instrumental prowess dictate the course of our program but hopefully marry these results to the pursuit of basic studies on flavor.

DR. R. T. MILNER (University of Illinois):

Are there any further questions? If not, I would like to thank all of the speakers and tell them how much we have enjoyed their papers.

Speaking in behalf of the audience, I would like to express the opinion of all of those who attended this meeting that we have gained much knowledge from it and that we would like to thank Dr. Mitchell and the others who were responsible for the arrangement of the meeting.

MITCHELL:

I want to express my appreciation to the N. R. C. Committee, especially to Dr. Mrak, without whose help we could not have arranged this conference, and most certainly to all of the speakers who have agreed and who have so willingly accepted the invitation to take part.

(The meeting adjourned at 3:45 P.M.).

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